"I think and think for months and years. Ninety-nine times, the conclusion is false. The hundredth time I am right."

Albert Einstein.

University of Alberta

PLANT PROTEIN LOCALIZATION BASED ON FREQUENT DISCRIMINATIVE SUBSEQUENCES AND PARTITION-BASED SUBSEQUENCES

by

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of **Master of Science**.

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To my respected parents, my nice grandmother
and my lovely wife, Mojdeh.

Abstract

Proteins, important macromolecules in living cells, are present in different locations within the cells, and a few are transported to the extracellular space. Each protein has a distinct function, and to fulfill that function they must be localized to the correct position in the cell. Therefore, discovering the localization of a protein helps analyze its role in the living cell. Extracellular proteins are of high importance due to their responsibility for vital functions such as nutrition acquisition, protection from pathogens, etc. Hence, characterizing these proteins and distinguishing them from intracellular proteins is of high interest to biologists. Nonetheless, this problem is very challenging because of the small number of available extracellular proteins[1].

This work focuses on extracellular and intracellular localizations. Using associative classifier we acquire a set of accurate, small and interpretable localization rules that can be used for further biological analysis. To classify proteins, which are linear sequences of amino acids, one should represent these by a set of features. In this work, the most frequent discriminative subsequences as well as partition-based subsequences are studied, i.e.,subsequences frequent in some partitions along protein sequences. The achievement of high F-Measure for predicting extracellular proteins shows high discrimination ability of the selected features.

[1]Our dataset contains only 127 extracellular proteins

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Chapter 1

Introduction

Proteins are one of the main structures of living cells that conduct different processes and functions in the cell. Proteins, at the simplest representation, are linear sequences of amino acids, and so far twenty standard amino acids have been identified in proteins.[1]. These amino acids are coded by twenty alphabetic characters as shown in Table 1.1. Therefore, proteins can be considered as character strings of different length varying from 41 amino acids or less, for a mitochondrial plant protein, to 3,705 or more, for an outer membrane plant protein. Biological experiments indicate that amino acid sequences encode information about protein structures, functions, localizations, etc .

Through genome sequencing projects, many datasets of raw biological sequences are collected, and are publicly available for researchers. With the interest to study genome sequences and the rapid growth of collected biological data, which adds complexity to the study and analysis of the sequences, there is a tendency toward utilizing computational algorithms and tools. This research addresses some algorithms to challenge one of the important problems about plant protein localizations.

1.1 Background, Problem Definition and Approach

One of the important problems in the biology community is the functional classification of proteins based on their structures, localizations, or other properties. In order for proteins to accomplish a specific function, they concentrate in differ-

[1]Unlike other amino acids that are present in biological proteins, Selenocysteine, the 21st amino acid, is inserted at a UGA codon in the context of other sequences within the mRNA.

	Name	Three-letter code	One-letter code
1	Alanine	Ala	A
2	Cysteine	Cys	C
3	Aspartic acid	Asp	D
4	Glutamic acid	Glu	E
5	Phenylalanine	Phe	F
6	Glycine	Gly	G
7	Histidine	His	H
8	Isoleucine	Ile	I
9	Lysine	Lys	K
10	Leucine	Leu	L
11	Methionine	Met	M
12	Asparagine	Asn	N
13	Proline	Pro	P
14	Glutamine	Gln	Q
15	Arginine	Arg	R
16	Serine	Ser	S
17	Threonine	Thr	T
18	Valine	Val	V
19	Tryptophan	Try	W
20	Tyrosine	Tyr	Y

Table 1.1: Table of natural amino acids [6]

ent locations inside the cell and sometimes they are transported to the extracellular space. The process through which proteins are routed to their subcellular localization sites is called protein sorting. The simplest sorting process happens in Gram-Positive prokaryotic cells. Prokaryotes are types of living organisms, mostly unicellular, that lack a cell nucleus or any other membrane-bound organelles [3]. In Gram-Positive prokaryotes, proteins are localized at only three intracellular sites and the extracellular space. Conversely, in eukaryotic cells, due to the presence of membrane-bound organelles, there are more localization sites, and consequently, protein sorting is more complex. Table 1.2 shows the different localization sites in different categories of cells. As this table shows, plant cells, with nine different localization sites inside the cells, are most complex in terms of protein localizations. These nine localization sites are generally referred to as intracellular (IC), while localization outside the cell is called extracellular (EC).

Protein sub-cellular localization is the key characteristic to study the function of

Category	Subcellular Localizations
Animal	nuc, end, gol, mit, pex, lys, cyt, mem, ext
Plant	nuc, end, gol, mit, pex, chl, vac, cyt, mem, ext
Fungi	nuc, end, gol, mit, pex, vac, cyt, mem, ext
Gram-Positive bacteria	cyt, wal, mem, ext
Gram-Negative bacteria	cyt, inn, per, wal, out, ext

Table 1.2: Subcellular localizations in different cells. Abbreviations are as follows: nuc (nuclear), end (endoplasmic reticulum), gol (golgi), mit (mitochondria), pex (peroxisomal), lys (lysosomal), cyt (cytoplasmic), mem (membrane), inn (inner membrance), out (outer membrance), chl (chloroplast), vac (vacuole), per (periplasmic), wal (cell wall), ext (extracellular) [46]

proteins. In plants, EC proteins are responsible for vital functions such as "nutrition acquisition, communication with other soil organisms, protection from pathogens, and resistance to disease and toxic metals" [48]. Therefore, they are of high importance for the cells and are a target of analysis in the biology community. Herein, we particularly focus on characterizing and predicting EC proteins by learning and classifying proteins to EC or IC locations.

Localization of proteins has been a research interest for bio-informaticians and machine learners for some time, but it is still a challenging problem mainly due to the lack of training data, and when data exists, to severe imbalance in the training data. Another difficulty is the identification of appropriate features in the data to accurately localize proteins. Some have used simple distribution of amino acids (i.e.,protein composition), subsequences, special signatures or combinations. In this research we start with studying frequent subsequences of proteins. The process of localization utilizes small subsequences of the protein to direct the protein to different localizations. Therefore, frequent subsequences, identified by our approach, might be of direct mechanistic significance. Based on frequent subsequences, we gradually evolve our feature mining algorithm by resolving the experimentally observed deficiencies of the older algorithms. Finally, we introduce the idea of taking advantage of partitioning sequences of amino acids and identifying the relevant partitions where some subsequences occur. These partitions appear to have discriminative power with regard to localization of proteins.

To do so, we transform the proteins that are originally represented as strings of

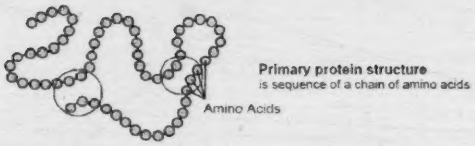


Figure 1.1: Structure of Protein [1]. The locations that may interact due to their close distance are circled.

amino acids into sets of frequent motifs extracted from these strings. Motifs are subsequences of amino acids that are frequently occurring in the collection. Then, protein sequences are partitioned in equal partitions[2] and each motif is labelled by the partition in which the motif frequently occurs. If a motif appears frequently in the same partition of some proteins with identical localizations, it is a valuable feature for the proteins of that localization. This is more complex than it appears, since each protein has to be expressed by some identified motifs, and identifying all partitions where motifs occur, given different partitioning intervals, is a hard problem. These features (i.e.,motif and partition pairs) are frequent subsequences associated with their discriminative partitions along the protein sequence, which we call Partition-Based Subsequence (or PBS). They constitute our input for our classifier which yielded better results than the state-of-the-art.

Our inspiration for introducing PBSs comes from the following observation. Proteins are of complicated shapes in 3-dimensional space. At this level, proteins of the same class may present higher similarity than at the simple level of amino acid sequences [36]. On the other hand, it is difficult to characterize the 3-D specifications of proteins. Discovering the special regions of protein structures where frequent subsequences appear most may encode significant information about the structure of proteins. For example, EC proteins may be folded such that some regions may have biochemical effects on each other due to their close distance (as Figure 1.1 illustrates). Such effects may cause special patterns to be formed in these regions. This is what motivated us to discover subsequence patterns that are frequent in special regions of protein sequences.

[2]The number of partitions is a user parameter.

Other than introducing PBS, a novel type of protein features, the prediction of EC proteins based these features is another contribution of this work. We use an associative classifier to predict EC proteins. The reason for our choice is that associative classifiers construct an interpretable rule-based model that can be used for further biological analysis. Figure 1.2 shows some examples of the human readable rules that our algorithm discovered from the data. Due to the popularity of support vector machines (SVM) [27] in the biological data mining field, we compare our results with those of SVM. As further experiments, the result of decision tree classifiers are also compared with that of associative classifiers.

If "GPPYCCS" appears in a protein sequence
$\Rightarrow$ The protein is extracellular.

If "SSSSSSS" appears in the first half of a protein sequence
$\Rightarrow$ The protein is intracellular.

Figure 1.2: Real examples of the human readable rules that our associative classifier discovered from the data.

As we mentioned earlier, due to the severe imbalance between the number of EC and IC proteins, and further, the small number of available training samples, a few proteins are not represented by any frequent and discriminative features with respect to different input parameters, and the prediction accuracy falls. Our approach tackles this problem to enhance the prediction by proposing a two-phase solution. First, a strong associative classifier with highly confident rules is constructed. In the second step, to classify those few proteins which cannot be classified by any of the associative rules, a nearest neighbor classifier based on edit distance [11] of protein sequences is utilized. Our experiments on a biologically verified dataset, show that the localization prediction based on associative classifier and PBS features strongly outperforms state-of-the-art algorithms with an EC prediction accuracy (F-Measure) of 89.06%. The recall and precision are respectively 89.79% and 88.31%.

1.2 Dissertation Organization

The rest of the thesis is organized as follows: Chapter 2 is a review of the related work. In Chapter 3 the history and evolution of solutions devised in this research are explained as well as the algorithm of mining discriminative frequent partition-based subsequences. In Chapter 4 the associative classifier for the special case of our problem is explained. Then a combined model of associative and nearest neighbor classifier is introduced. Experimental results are discussed in Chapter 5, and finally Chapter 6 concludes the thesis and present the future work.

Henceforth, for convenience we refer to Intracellular, Extracellular and Partition-Based Subsequence as IC, EC and PBS respectively.

Chapter 2

Related Work

This chapter studies the state-of-the-art in predicting protein localizations. Section 2.1 reviews the challenges of this problem and the approaches that are investigated by researchers. Related to our approach, which is based on frequent subsequences of proteins, Section 2.2 presents different solutions for frequent subsequence mining.

2.1 Work Related to Protein Subcellular Localization

Several approaches have been proposed to predict different protein localizations. These approaches differ in the features and the classification methods they have used. Generally these works can be grouped in five different categories.

2.1.1 Prediction Based on N-Terminal Sorting Signals

Sorting or targeting signals are the pieces of information, encoded in a chain of amino acid residues, that enable the cellular transport machinery to direct proteins to inside or outside the cell. In other words, sorting signals are "short subsequences of approximately 3 to 70 amino acids and can be identified by looking at the primary protein sequence" [46]. It has been claimed that for proteins targeting the secretory pathway, mitochondria, and chloroplasts, sorting depends on the signals that are found at the N-terminal extension of protein sequences. N-terminal signals (presequences or peptides) are often cleaved off the mature protein upon arrival at the proper localization site. Following is the list of these signals:

- Signal Peptides (SPs): They signal proteins to traverse the membrane of the rough endoplasmic reticulum. They control the entry of proteins to the secretory pathway and are cleaved off while the protein is transported through the membrane [16]. "The most well conserved motif of the SPs is the presence of a small and neutral amino acid at positions -1 and -3 relative to the cleavage site" [29].

- Mitochondrial Targetting Peptides (mTPs), which control the transportation to mitochondria. In mTPs, the amino acids "Arg, Ala and Ser are over-represented while negatively charged amino acid residues (Asp and Glu) are rare. Only weak consensus sequences have been found, the most prominent being a conserved Arg in position -2 or -3 relative to the mitochondrial processing peptidase (MPP) cleavage site" [29].

- Chloroplast Transit Peptides (cPTs), that direct most nuclearly encoded chloroplast proteins to the chloroplast area. cPTs of different proteins vary in length and sequence, however, they have a rich content of hydroxylated and low content of acidic residues [28].

According to these specific features of signals, different algorithms have been proposed to identify the signals and their cleavage sites, and consequently predict the localization of proteins using their identified sorting signals. MitoProtII [14] has performed discriminant analysis to recognize mTPs. It has achieved a mitochondrial prediction with 80% recall and 47% precision. In an effort to increase the accuracy of mitochondrial prediction, Bender et al. [2] has achieved the recall of 94% and precision of 68% based on a neural network approach. For the identification of SPs and cTPs, neural networks are utilized by SignalP [16] and ChloroP [28]. The highest reported accuracy of SignalP is 83.7% for correct identification of signal peptide cleavage sites on E.coli data [1]. The accuracy of ChloroP for identifying sequences as cTP or non-cTP is 88%.

The same group that devised SignalP and ChloroP has proposed TargetP [29] by integrating their previous approaches in order to predict four different localizations:

[1] E. coli signal peptides are different from eukaryotic signal peptide

chloroplast, mitochondrial, extracellular and other localizations. TargetP has been able to correctly predict localizations with the overall accuracy of 85% for plant proteins and 90% for non-plant proteins.

2.1.2 Prediction Based on Protein Annotations

In SWISS-PROT sequence database, only a few proteins are labelled with their subcellular localization, but there are functional annotations for many proteins. These textual annotations are generated automatically with limited human interaction [32]. The keywords of the textual annotations can be a good source based on which localization of the proteins can be inferred. The approaches in this category perform lexical analysis to extract keywords from the textual annotations of homologous proteins, and then apply classification algorithms on the keyword-based feature datasets. It is similar to what happens in text categorization problems where unknown documents are assigned some predefined labels based on their lexical similarity to the documents that are already labelled. Many learning methods have been used for text categorization including nearest neighbor and K-Nearest neighbor classifier [44, 40], multivariate regression models [17], probabilistic Bayesian models [12], linear least square fit [45], etc .

Based on a similarity-based approach, LOCkey [33] infers the localization of a protein by categorizing its textual annotation into a set of subcellular localizations. The approach of LOCkey is as follows:

- A set of proteins with known localization are collected. Then, from the textual annotation of each protein (available in SWISS-PROT), keywords are extracted.
- Keywords of homologous sequences are merged. A feature reduction is then applied to purify the keywords.
- Using the complete set of keywords, proteins are represented as binary vectors (presence or absence of keywords). This data set is called "trusted vector set".

- For a protein U with unknown localization, all its keywords (out of the informative keywords of the trusted vectors) are specified. U is then represented by a binary vector $V(U)$ similar to the trusted vectors.

- All sub-vectors of $V(U)$ are generated, i.e.,all the possible combinations of the keywords of U. For example, if there are four keywords and $V(U) =< 0111 >$, subvectors of $V(U)$ are $< 0001 >, < 0010 >, < 0100 >, < 0011 >, < 0101 >, < 0110 >$ and $< 0111 >$. The subvector that yielded the best matching with one of the trusted vectors takes the localization label of that trusted vector as the localization of U. Selecting the best matching is based on minimizing an entropy-based objective function.

LOCkey considers ten different localizations for proteins and has achieved the accuracy of 87%.

Proteome Analyst (PA-Sub) [46], one of the prominent state-of-the-art algorithms, is also based on the lexical analysis. Unlike LOCkey, which uses entropy-based techniques to infer the localizations, the authors of PA-Sub [13] have applied several machine learning techniques such as K-nearest neighbor, naïve bayes, artificial neural network and support vector machine. PA-SUB has achieved the overall accuracy of about 93% on plant protein localization. However, for some classification issues, it has excluded almost 2% of the data from evaluation. The average F-Measure of PA-Sub in predicting EC proteins on exactly the same proteins of our test dataset is 83.77% which is more than 5% below the F-Measure of our approach.

2.1.3 Prediction Based on Amino Acid Composition

To biologists, the distribution of amino acids in proteins can be a meaningful feature. In this context, a protein is represented by the relative frequency of the twenty amino acids in the sequence of that protein. This representation is called "Amino Acid Composition" of a protein and results in a 20-dimensional dataset. Figure 2.1 illustrates such a representation for an EC protein.

Nakashima et al. [15] have found discrimination between EC and IC proteins by amino acid compositions and residue-pair frequencies. Based on statistical anal-

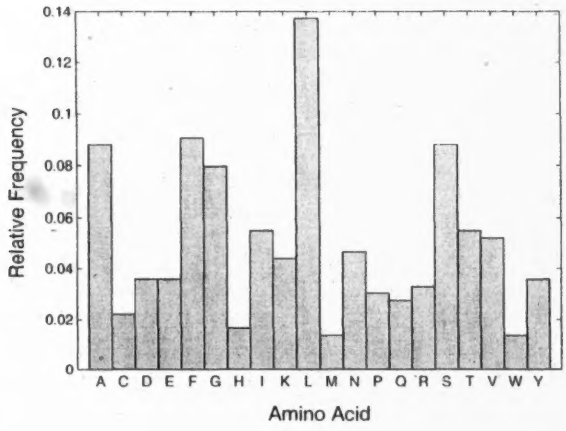


Figure 2.1: Histogram representation of the amino acid composition of an extracellular protein.

yses, they have succeeded in correctly classifying 84% of EC proteins and 88% of IC proteins. They did not report the F-Measure of their model. However, with a simple calculation and assuming that the number of EC proteins in their data is smaller than that of IC proteins, the F-Measure [2] of their model is at most 85.71%.

Prot-Lock proposed by Cedano et al. [18] uses the same representation of proteins to learn five different localizations by means of Mahalanobis distance of proteins. The statistical-based approach of Chou et al. [9], which applies a covariant discriminant algorithm, outperforms Prot-Lock with 79.9% overall accuracy.

To predict three locations in prokaryotic proteins and four locations in eukaryotic proteins (including EC) from amino acid compositions, neural networks [5], Markov chain models [47] and SVM [38] have been used by different researchers. Among them, the SVM-based method has attained the highest overall accuracy of 91.4% on prokaryotic and 79.4% on eukaryotic organisms.

2.1.4 Prediction Based on Frequent Subsequences

Frequent subsequences within proteins are other features used for subcellular localization. A *frequent subsequence* is a consecutive series of amino acids that appear

[2] A harmonic average of recall and precision

in more than a certain number of proteins of a specific class. In this context, proteins are represented in terms of frequent subsequences they contain. Zaïane et al. [48] used such features and applied SVM and boosting methods to predict EC localization. Their highest F-Measure is 80.4% when SVM is used. In another effort, they have used discriminative frequent sequential patterns as rules [48]. A *frequent sequential* pattern is of the form $*X_1 * X_2 * ... * X_n*$ where X_i is a frequent subsequence and $*$ represents a variable-length-don't-care. The same method for localizing Outer Membrane (OM) proteins has been used by She et al. [36]. Their rule-based classifier "has very good performance in terms of OM class precision (well over 90%), however, the corresponding recall is low (around 40%)" [36].

2.1.5 Prediction Based on Integrative Approaches

The last category of approaches is the combination of different methods. The previous work on predicting extracellular plant protein localization, by Zaïane et al. [48], applies a boosting algorithm on proteins where each protein is represented by a combination of its frequent subsequences and its amino acid composition. Their approach has reached an F-Measure of 83.1%, which is outperformed by the 5% higher F-Measure of our method. With SVM as the learning algorithm, Li and Liu [42] predict protein locations by combining N-terminal signals and amino acid compositions. Their highest achievement is 91.9% overall accuracy on non-plant proteins. Höglund et al. have achieved the overall accuracy of more than 74% by combining N-terminal signals, amino acid compositions and sequence motifs [4]. PSORT [23], probably the most complete tool for predicting many different localization sites, integrates various statistical methods and classification algorithms. However, its overall accuracy is less than 66%.

2.1.6 Challenges and Limitations of the State-of-the-Art Methods

What motivates us to proceed with the research idea of this dissertation is the presence of some limitations in the algorithms mentioned above, specially when they are considered for the case of EC localization prediction. The deficiency of these

algorithms highlights the need for our research and the suitability of the algorithm proposed in this research. In the following, the limitations of the-state-of-the-art methods are listed:

- Because of the important role of EC proteins in plants, it is valuable for biologists to access solutions for predicting such proteins. Therefore, our work particularly focuses on discriminating EC and IC proteins, while most of the existing algorithms are not well-devised and suitable for this purpose.

- Some of the state-of-the-art algorithms suffer from either low precision or low recall. However, most time the overall classification accuracy of the models is reported which is often a higher measure. For example, in the case of our problem, a classifier that always classifies as IC will have a recall and F-Measure of zero while the overall accuracy of such a classifier is 96% because EC proteins are only 4% of the entire plant protein dataset. Therefore, overall accuracy is not as informative as F-Measure. Our work tries to increase both recall and precision at the same time so that both a high F-measure and high overall accuray is achieved.

- Our approach tackles a hard and challenging binary classification in which there is a high imbalance between the number of samples of the two classes (EC and IC). Handling such an imbalance is not a matter of attention in many of the approaches.

- In most cases, the algorithms fail to present a justification of their prediction. A biologist may not rely on the results of a neural network or an SVM classifier if they need some tangible and understandable biological facts extracted by the prediction models. Using the associative classifier in our research helps to acquire not a black-box classifier but a set of accurate, small and interpretable localization rules that can be used for further biological analyses. Moreover, our secondary classifier, nearest neighbor classifier, is biologically justified when the distance of proteins is based on edit distance. The reason is that amino acid deletions, insertions, substitutions (mutations) along pro-

tein sequences happen through biochemical processes, and these three are the permitted operations in the computation of edit distance.

- Partition-Based Subsequence (PBS) is a novel type of protein features that has never been proposed before. By using PBSs, we probably indirectly exploit information about the folded structures of proteins in prediction, something that is not considered in the mentioned works.

- Some algorithms are the solutions to specific problems and cannot be extended to a complete localization problem in which learning all the possible localizations is targetted. For example, TargetP cannot classify beyond extracellular, mitochondria and chloroplast, as all proteins that cannot fall in any of the three localization classes are classified as "other". The specific localizations in "other" proteins cannot be learnt by the approach of TargetP. In contrast, our approach, although not experimentally confirmed yet for the complete localization prediction, has the potential to tackle multi-class localization problems (Refer to Future Work section).

2.2 Work Related to Frequent Subsequence Mining

There are different approaches to mine frequent subsequences of a given length and a minimum frequency or support ($MinSup$). A naïve solution is shown in Figure 2.2 that recursively builds subsequences and counts their support. This algorithm can handle only small datasets that can fit in main memory. It needs a large memory in the case of mining long subsequences when the recursive function goes too far deep.

As another solution for subsequence mining, an APriori-based approach is proposed. Frequent subsequences meet the apriori property, i.e.,if a subsequence $S[1...n]$ of length n is frequent, subsequences $S[1...n-1]$ and $S[2...n]$ are frequent too. Therefore, this algorithm first mines frequent subsequences of length 1. Inductively, to mine frequent subsequences of length $n+1$, subsequences $S[1...n]$ and $Q[1...n]$ of length n where $S[2...n] = Q[1...n-1]$ combine and create a candidate

```
minFreq: minimum frequency for a subsequence
minLen: minimum length of a subsequence
maxLen: maximum length of a subsequence
protIDs: set of IDs of all proteins

Main function call:
    MINE-FREQUENT-SUBSEQ({}, protIDs)
```

```
MINE-FREQUENT-SUBSEQ(seq: Sequence, prots: Set of IDs of proteins containing seq)
1   foreach x ∈ {AminoAcids}
2       newSeq ← append x to seq
3       newProts ← a subset of prots containing newSeq
4       if size(newProts) ≥ minFreq ∧ Length(newSeq) ≤ maxLen
5           if minLen ≤ Length(newSeq)
6               print(newSeq, newProts)
7           end
8           MINE-FREQUENT-SUBSEQ(newSeq, newProts)
9       end
10  end
```

Figure 2.2: A naïve algorithm for mining frequent subsequences.

subsequence C of length $n+1$, where $C = concatenation(S, Q[n])$. Algorithm 1 shows the details and Figure 2.3 illustrates the candidate generation.

The algorithms for sequential pattern mining can also be modified to mine frequent subsequences. In the context of sequential pattern mining, a sequence is an ordered list of itemsets and is denoted by $< S_1...S_n >$ where S_i is an itemset. For example, the chronological sequence of shopped items of a specific customer from a store can be such a sequence. In this context, a sequence $< A_1...A_n >$ is "contained in" another sequence $< B_1...B_m >$ if there exists n integers $i_1 < i_2 < ... < i_n$ such that $A_1 \subseteq B_{i_1}, ..., A_n \subseteq B_{i_n}$ [31]. Given a minimum support $MinSup$, if a sequence S is contained in more than a certain number (with regard to $MinSup$) of input sequences, then S is a sequential pattern. There has been significant work on mining sequential patterns such as GSP [37], SPADE [21], PrefixSpan [19], etc . In the case of our problem, if each amino acid is considered an itemset with size 1, i.e.,that amino acid is the only item in that itemset, then each protein can be considered as one of the so-called sequences. In the above definition of "containment", if this constraint is imposed that the n integers $i_1, i_2, ..., i_n$ are consecutive integers, then a

Algorithm 1: Mining frequent subsequences, an Apriori-based approach

input : *MinSup*

```
// MinFreq:  Minimum frequency for a subsequence
// C_k:  Candidate subsequences of length k
// L_k:  Frequent subsequences of length k
```

output: Set of Frequent Subsequences

```
begin
    L_1 ← {frequent single characters}
    k ← 1
    while L_k is not empty do
        C_{k+1} ← Candidates generated from L_k
        foreach Subsequence S in dataset do
            Increment the count of all candidates in C_{k+1} that are a
            subsequence of S
        end
        L_{k+1} ← Candidates in C_{k+1} with frequency ≥ MinFreq
    end
    return ⋃_k L_k
end
```

sequential pattern is exactly a frequent subsequence.

Among all the different solutions for frequent subsequence mining, Generalized Suffix Tree (GST) is what we used in our research. It is one of the most efficient methods and its code is publicly available [20]. The suffix tree of a single string is a tree structure that stores all the suffixes of that string. GST is a more general structure to store all the suffixes of a set of strings.

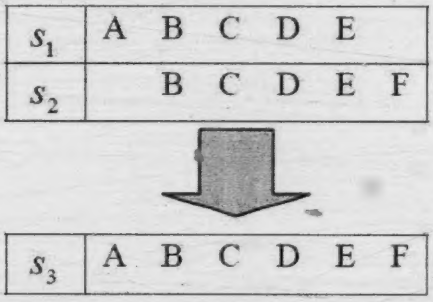


Figure 2.3: An example of candidate generation. S_1 and S_2, two frequent subsequences of length 5, generate S_3, a candidate subsequence of length 6

Figure 2.4 shows the GST of three strings. As this figure shows, edges are labelled with character strings and leaf nodes (square nodes) hold an index. The concatenation of edge labels from the root to a leaf node with index i is a suffix of the ith string. Each internal node (circular node) stores the frequency of the substring which is constructed by concatenating the edge labels from the root to that node. For example, tracking "K" from the root ends at a circular node and two square leaf nodes. The value 3 in the circular node is the frequency of the subsequence "K", and the indexes 1 and 3 in the square nodes show that the 1st and 3rd strings has "K" as a suffix.

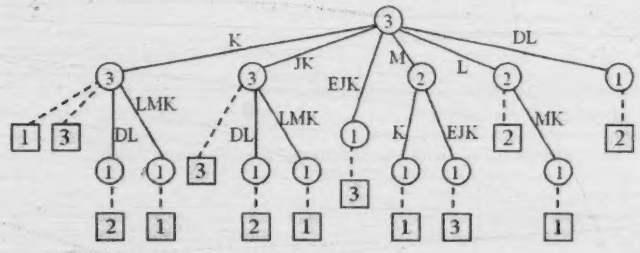


Figure 2.4: The GST of three strings: 1) JKLMK, 2) JKDL, 3) MEJK

Constructing GST requires the concatenation of input strings with some separator symbols between them and then making a suffix tree on this long string [11]. There are efficient algorithms for online construction of GST in linear time [11]. After the GST is constructed, frequent subsequences are mined through a single traversal of the tree [20]. Although constructing GST takes $O(n)$ time [20], the concatenation of all proteins results in an enormously long string. Tata et al. [39] have proposed practical solutions for GST construction.

Chapter 3

Protein Feature Extraction

A protein, in a simple 1-dimensional representation, is a sequence of amino acids. It needs to be represented by a set of features in order to be in a suitable format for learning algorithms. A feature dataset can be in a relational or transactional format. In transactional format, a protein is in the form of a set of specific features, e.g., frequent subsequences, extracted from that protein. These sets are not necessarily of the same size. In order to transform such data to a relational format, data is organized in a table structure where each column corresponds to a feature, and rows represent proteins. In this format, a protein is assigned a value of 1 at a column if the protein possesses the feature related to that column; otherwise, 0.

If the original format of data is relational, e.g., the case where proteins are represented by their amino acid compositions, a transactional format can be simply obtained by discretizing all the continuous attributes, assiging unique codes to each attribute-value pair, and representing each protein by the set of the codes of their attribute-value pairs.

Since in this research, an associative classifier is used for learning and predicting the protein localizations, and associative classifiers deal with data in transactional format, the feature datasets should be transactional. To find out whether a feature set is suitable for localization prediction, an associative classifier is applied on the feature dataset. If a high prediction accuracy is achieved, the extracted features are satisfactory; otherwise, other types of features should be studied. It is possible for a feature dataset not to be well learnt by associative classifiers while another classifier could learn the same dataset better. However, the accuracy of associative classifier

is our main criterion for the suitability of extracted features since our ultimate goal is building an interpretable and accurate model using strongly confident associative rules.

In this work, we focus on two types of features:

- Frequent subsequences or FS.
- Frequent Partition-Based Subsequences or PBS, subsequences that are frequent in some discovered partitions of protein sequences.

The reason why our focus is on subsequence-based features is based on the following observations:

- "Common subsequences among related proteins may perform similar functions via related biochemical mechanisms" [36] and are of great interest to biologists.
- "Frequent subsequences capture local similarity that may relate to important functional or structural information of extracellular proteins" [43].

Therefore, it is expected that frequent subsequences of proteins or "motifs" can better discriminate proteins of different localization.

The following sections, elaborate on these features. Section 3.1 is a history of the algorithms we tried to represent proteins by their FS features. At each step, a deficiency of the algorithm is observed and the next algorithm aims at resolving it. The series of different approaches are evolved until the algorithm for mining PBSs is devised. The explanation about PBSs is brought in Section 3.2

3.1 History of Frequent-Subsequence-Based Feature Mining Algorithms

As discussed in Chapter 2, there are different approaches to mine frequent subsequences. However, because of the availability and efficiency of the GST-based subsequence mining code [20], this algorithm is used. The important parameters of this algorithm are:

- $MinSup$: The minimum support of a subsequence to be frequent. Support of a subsequence S is the fraction of protein sequences that contain S
- $MinLen$: The required minimum length of a subsequence

- *MaxLen*: The required maximum length of a subsequence

After mining frequent subsequences, each protein is represented by the frequent subsequences it contains, and a transactional feature dataset is built. Although building such a feature dataset seems straightforward, our experiments on the plant protein dataset showed many unexpected problems and difficulties. These problems can be summarized in three categories:

1. The first and main problem is the imbalance between the size of EC and IC proteins (127 vs. 3,022). Because of the diversity in the larger group, it is very likely for the proteins in that group to contain the frequent subsequences of the smaller group. It causes the subsequences of EC proteins to be less distinctive, i.e., features from the rare EC group are not frequent enough to be captured.

2. Another problem is that the resulted feature dataset may contain large transactions of hundreds of items. It is a serious problem for an associative classifier to handle large transactions. In this case, the number of outputted rules is so huge that processing them is sometimes impossible with the available equipment. Long transactions are generated when mined subsequences are so much frequent that each protein contains a significant number of them. Thus, any subset of subsequences, which is potentially a rule, is probably frequent. Therefore, a control is required over the length of the resulted transactions during the whole process of frequent subsequence mining.

3. The last problem is the existence of *silent proteins* in the feature datasets. Silent is referred to a protein that does not contain any of the frequent subsequences that were extracted, i.e.,all their subsequences have low frequency. On the other hand, lower frequencies lead to mining a huge number of subsequences, which is hard to process.

Facing all these problems and attempting to resolve them, the algorithm of building a subsequence-based feature dataset gets gradually evolved by different modifications. The following sections explain different steps of the evolution of our algorithm.

3.1.1 Class-Specific Subsequence Mining

In our dataset, 127 proteins are EC and 3,022 proteins are IC. It means that EC proteins represent almost only 4% of the dataset. Thus, in mining frequent subsequences, if $MinSup$ is more than 4%, no subsequence specific to EC proteins is found. On the other hand, $MinSup$ of less than 4% is very low for the IC class with abundant proteins, and thus a huge number of frequent subsequences are generated which makes the later processings complex.

To solve this problem, subsequence mining is no longer done on the whole protein set. Instead, it is done separately on the proteins of each class. This is a fair modification because at a fixed input of $MinSup$, the frequency threshold is proportional to the size of each dataset. Thus, at different ranges of $MinSup$, frequent subsequences of each class have the chance to emerge.

3.1.2 M-Most Frequent Subsequences

Given a $MinSup$ by which few or no silent proteins exist, such a large set of motifs are found that the average size of transactions in the feature dataset becomes very large [1], which is hard for associative classifiers to process. Therefore, there should be a limit on the number of mined motifs. A parameter "M" that selects M most frequent subsequences can be a simple modification. A good selection of short motifs, i.e.,length 3 or 4, results in an accurate classifier, however, there are some drawbacks to this model. First, these short motifs are common in both classes. Thus, they are not class-distinguishing by their own, and it is the association of motifs that discriminates classes. Moreover, this model lacks flexibility in the length of the motifs. With motifs of higher length, many silent proteins appear because some proteins contain the M-most frequent subsequences even for large values of M such as 1000. Hence, in the feature dataset, this particular set of expressed proteins becomes long transactions of size around M, while the rest of the proteins are silent.

[1] Experimentally (in the environment that is explained in the experimental study section), if the average size of transactions exceeds 30, the number of rules produced by associative classifier and the execution time fall in the scale of milions and days respectively.

Henceforth, we refer to motifs of length less than 5 as *SHORT* and higher length motifs as *LONG*. In the future algorithms we focus on mining long motifs.

3.1.3 M-Most Frequent Maximal Subsequences

Reviewing the feature set of M-Most frequent sequences reveals that if subsequence S is in the feature set, all the subsequences of S that satisfy *MinLen* requirement, are also in the set as they can have higher frequency than that of S. Further, if a protein includes S, there is no advantage in presenting the protein with subsequences of S. Those subsequences only occupy wasted space in M-Most frequent subsequence (FS) set while they carry no information, i.e.,are redundant. For example, consider the following features:

Subsequence (in M-Most FS set)	**Proteins containing Subsequence**
'ABCD'	P_1, P_2, P_3, P_4
'ABC'	P_1, P_2, P_3, P_4
'AB'	P_1, P_2, P_3, P_4
'MNOP'	P_1, P_2, P_3, P_4

Table 3.1: An example of motifs carrying no additional information.

In the feature dataset, each of the proteins P_1, P_2, P_3 and P_4 is a transaction with items 'ABCD', 'ABC', 'AB', ... while selecting only 'ABCD' among these three suffices. This modification makes the feature dataset smaller and opens space in the M-most FS set for the silent proteins to introduce their motifs to the set.

It can be even more generalized to totally different motifs that come from the same set of proteins, i.e.,if S and T are two different motifs both contained in the same set of proteins P, then S and T can be replaced by the new symbol U where U={S, T} represents proteins in P. For instance, in table 3.1, instead of features 'ABCD' and 'MNOP', feature U={'ABCD', 'MNOP'} can represent proteins P_1, P_2, P_3 and P_4. Totally Table 3.1 can be reduced to only one feature U, and three spots are freed for more motifs (possibly from silent proteins).

Unfortunately we could not take advantage of this modification because still M-most motifs cannot re-express all the proteins, and many silent proteins, specially in IC class, are still observed.

3.1.4 N-Most Discriminative Motifs

Instead of filtering motifs by selecting the M-most frequent ones, they can be filtered by selecting the N-most discriminant motifs. Given a $MinSup$ and $MinLen$, all motifs are mined separately from each class. If a motif is frequent in one class and also appears in another class, it is not considered discriminant. Based on this criterion, *coincidence degree* of a subsequence of a class is defined as an indicator of the appearance of that subsequence in the opposite class. In this algorithm, if a motif is discriminant, its coincidence degree is zero; otherwise, one. Of the discriminant motifs, N most frequent ones are selected to make the feature dataset. If they are less than N, we have to select out of the non-discriminant motifs.

This modification still suffers from silent proteins. Even if the value of N is set close to the whole number of mined motifs silent proteins will show up (even at low $MinSup$)

3.1.5 N-Most Discriminative Motifs Based on IC Localizations

Based on the fact that proteins of a same localization present higher similarity, it might be a good idea not to put all the intracellular proteins in only one group of IC. They can be grouped as the proteins of nucleus, mitochondria, etc . In this way, features of each type of intracellular proteins are independently captured, and it is expected that more proteins become expressed. In the previous algorithm, if a motif of a class does not appear in the other class, it is considered discriminant and receives a coincidence degree of 0, otherwise 1. Here in this algorithm, instead of mining motifs of EC and IC proteins, motifs of each of the 9 IC localizations (mentioned in Section 1.1) and the EC localization are mined separately and the coincidence degree of each motif, which is now an integer between 0 and 9, is computed. Afterwards, all the motifs derived from proteins of classes other than EC are collected as the motif set of IC class. Then N-most discriminant motifs (having lowest coincidence degrees) are selected from the motif set of each class and the feature dataset is constructed based on the union of the two motif sets. The result of this algorithm is more frustrating because many of IC proteins appear silent. The

reason is that at some IC localizations, a few proteins exist whose frequent subsequences can not be well learnt. For example, peroxisomal contains only 29 proteins which is a very few number of training samples, and learning the pattern of peroxisomal proteins based on frequent subsequences can not be accurate. Moreover, proteins of the nine IC classes are very similar in terms of their structure and sequences. Therefore, the frequency of IC frequent motifs is accumulated over the nine different classes. Dividing IC proteins into 9 separate classes, breaks down the frequency of those motifs and they fail to reach the minimum frequency in this algorithm. Based on this experience and the inferences, the next algorithms consider all proteins of the nine IC localizations as a whole.

Protein ID	**Symbolic Presentation of The Features**
P_1	* * * } Small number of motifs (short transaction-like proteins)
...	...
P_{1998}	* *
P_{1999}	* } Large number of motifs representing around 330 IC(chloroplast) proteins (long transaction-like proteins)
P_{2000}	* * * * * * * * * * * * * * * * * * *
...	...
P_{2331}	* *
P_{2332}	* * * * * * * * * * * * * * * * * * *
P_{2333}	* } Small number of motifs (short transaction-like proteins)
...	...
P_{3149}	* *

Figure 3.1: The length of proteins in the feature dataset where $MinSup$ is appropriate enough for all proteins to be expressed by at least one motif

3.1.6 Dynamic Support-Feature Minimization

At this point, we found out that some proteins own very rare or unique subsequences. In other words, they have no shared subsequences with other proteins. In order to prevent them from silence, the minimum frequecy of 1 or 2 is required in which an exploding number of subsequences are retrieved. The investigation in the dataset revealed that almost 10% of IC proteins (330 chloroplast proteins) contain almost all the motifs of 5% support. Therefore, if $MinSup$ is set to a value that

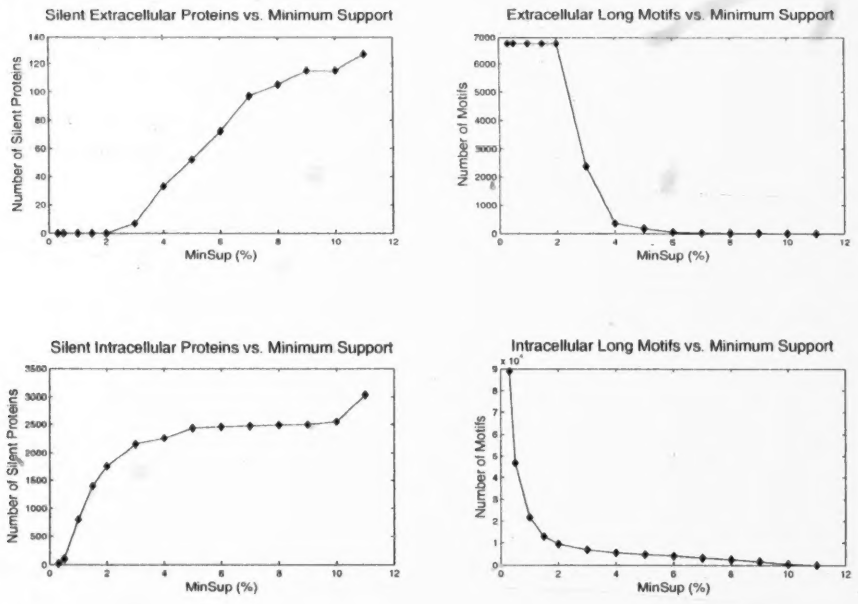


Figure 3.2: The effect of minimum support on the number of mined long motifs and silent proteins in EC and IC classes

prevents silent proteins, the feature dataset looks like Figure 3.1 where chloroplast proteins are very long transactions with a lot of motifs. These long transactions are a serious problem in associative rule mining for classification and generate too many rules. To overcome this problem, the two-phase Algorithm 2 seems a possible solution.

This algorithm is expected to decrease the number of motifs as well as the length of transactions. However, there are some drawbacks to the algorithm. First, two different $MinSup$'s for the two classes make unequal situations for the two classes. Second, even if we accept this unequal situation, decreasing $MinSup$ at each step results in mining more motifs to the extent that finally a tremendous number of motifs are extracted just to prevent silent proteins. Figure 3.2 shows a comparison of silent proteins and the number of mined long motifs for different $MinSup$ values. Based on the experiments, even at $MinSup = 0.5\%$, there are still 99 silent IC proteins although more than 46,000 motifs are extracted.

Algorithm 2: Dynamic Support - Feature Minimization

input : $MinSup_{EC}$, $MinSup_{IC}$
(Initial minimum support for motifs of EC and IC proteins)
output: *features*

```
begin
    //Phase1 (Finding the best values of MinSup for each class):
    foreach Class c ∈ {EC, IC} do
        while There are silent proteins of class c do
            Decrease MinSup_c by some step
            Mine frequent motifs among proteins of class c
        end
    end
    // At this point, it is expected no silent protein remains
    // Phase2 (Filtering motifs for each class separately):
    foreach Class c ∈ {EC, IC} do
        Sort the list of motifs of class c in the increasing order of their coincidence degrees (Dicriminative motifs first)
        // Selecting most dicriminative motifs:
        From the begining of the sorted list pick motifs until all the proteins of class c are expressend (no silent proteins) and remove the rest of motifs
        Create the feature dataset of proteins of class c based on the selected motifs
        AvgLen ← Average length of transactions
        foreach Transaction-like protein p do
            if length of p ≤ AvgLen then
                Label p as short
            end
            else
                Label p as long
            end
        end
        Decreasingly Sort the list of motifs based on the times they appear in short proteins (Motifs contributing to short transactions first)
        // Minimizing the length of transaction-like proteins:
        From the begining of the sorted list pick motifs until all the proteins of class c are expressend (no silent proteins) and remove the rest of motifs
    end
end
```

Based on Figure 3.2, the fewer silent proteins, the more motifs. Hence, a compressing data structure should be used to store motifs and the Id of their proteins in order for further processing. A "Trie" may be the best candidate of such a structure. "Trie" is a tree structure where the internal nodes hold alphabetic characters and the nodes of each level are alphabetically sorted. Trie is mainly used to store a dictionary of words with a fast search possibility. A path from the root to a leaf constitutes a word. In our case, if 'X' is a motif of length n, it corresponds to a node at level n. This node maintains the set S of IDs of the proteins that contain the corresponding motif. If 'Xa' is also a motif where 'a' is just a single character (one amino acid), the corresponding node contains set T of IDs such that $T \subseteq S$. Thus, the members of T should be removed from S to avoid repetitive information. In this way, lots of memory is saved.

All of these problems motivate for alternative solutions to be considered.

3.1.7 Dynamic Support - Rare Motif Detection

According to our previous experiments, it was found out that some proteins do not contain frequent motifs, i.e.,remain silent. All their motifs are unique to them or very rare. These rare motifs can be considered important as they identify proteins with uncommon or specific patterns, probably with special properties. As we explained in the previous section, setting $MinSup$ to small values for mining rare motifs is not appropriate as it makes normal proteins expressed with their frequent as well as rare motifs. Therefore, extra information will be produced. Algorithm 2, mentioned in the previous secion, can be simply modified such that frequent motifs and useful rare motifs are extracted separately. In this modification, an initial $MinSup$ (to be the criterion of "frequentness") is given to each class and frequent motifs are mined. Then for each class, the proteins are separated into two sets: those that are expressed by the frequent motifs, S_1 and those that are not, S_2. Now algorithm 2 is applied to all proteins, but of the newly generated motifs, only those representing at least one protein of set S_2 are kept and the rest are ignored. Therefore, if a protein is already expressed by some motif, it is not re-expressed by newly mined motifs (with lower supports) and many useless motifs are filtered.

Figure 3.2 helps to set initial $MinSup$ of each class. In an experiment, the initial $MinSup$ of 4% and 10% were selected for motifs of EC and IC class respectively. In mining rare motifs, it was proven that the minimum absolute frequency of 2 is needed for some proteins to be expressed; otherwise, they remain silent. In this case, although rare motifs only from set S2 are supposed to be extracted, more than 50,000 motifs with absolute support of 2 or more appear.

3.1.8 Dynamic Support - Most Discriminative Frequent Motif

To filter more motifs, the previous algorithm can be modified as follows:

Of the different motifs that express a protein P, P has to pick only the motif with the highest score. Score can be frequency, length, or a weighted sum of them, etc . When a new motif is mined, all the proteins containing that motif compare its score with the score of the motif they have already had. A protein replaces its older motif with the new one if a higher score is obtained.

This algorithm does not guarantee that the length of each transaction in the feature dataset is 1. For example, a protein P_1 may contain motifs M_1 and M_2 but only M_1 is its best motif. On the other side, M_2 may be the best motif of another protein P_2. Although P_1 and P_2 each select one motif, but in the end, since both motifs are kept in the mined feature set, P_1 has to be also represented by M_2. Due to this fact, long transactions still persist. A bigger problem is that silent proteins are avoided in return for decreasing $MinSup$ to very small values. It causes some proteins to be only expressed by very rare motifs. Later, an associative classifier generates no rule to classify such proteins because it finds frequent rules based on frequent motifs. In the next section (3.1.9) we explain and solve this problem.

3.1.9 N-Best (Longest) Motifs

Since this project develops a classification model, and a classifier represents a general (frequent) pattern of data, we could state that only general (frequent) motifs are needed. Although decreasing $MinSup$ lessens the number of silent proteins, rare motifs cannot contribute to classification rules, which are frequently observed relations between data and classes. Later on, test proteins expressed by rare motifs

cannot be classified. We should adopt the fact that there is no frequent and long motif for some proteins (silent proteins) and rare long motifs seem useless at the end. On the other hand, we are interested in long motifs although frequent short motifs can express all proteins, as mentioned previously in section 3.1.2. These two ambitions (high length and frequency) are considered in the following approach:

Input an integer N and only one desirable $MinSup$ value (the same $MinSup$ for both EC and IC). Throughout the algorithm, $MinSup$ is never changed, but the length of the motifs to be mined decreases until no silent protein remains. Each protein is supposed to be expressed with at most N best motifs. Selecting the best motifs is based on a score that is defined as a function of length, frequency, number of occurrences in the other class, etc . The simplest way is to assign higher scores to longer motifs and if motifs are equally long, to those that are more discriminant (fewer occurrences in the other class). The details are shown in Algorithm 3.

In this algorithm, proteins start selecting their motifs from the longest ones. Length is a good selection criterion because longer motifs may contain more information than short ones. Moreover, "with the alphabet of only 20 amino acids, it is likely that very short subsequences will occur in sequences of both classes and such subsequences are non-discriminative with regard to classification" [36].

Based on this algorithm, a protein is marked as **NeedingMotif** when it is not yet expressed by N motifs. To find a setting for N, if it is set to 1, then only the proteins that are completely silent are marked as **NeedingMotif**. Experimentally we observed that all the proteins can be expressed by frequent motifs not shorter than 3.

As compared to the previous algorithms, this algorithm has a more reliable strategy to find discriminative motifs. Previously, coincidence degree of only 0 or 1 was assigned to motifs to indicate if it is discriminant. Then, motifs were filtered based on this degree. It sounds unfair because if a motif is very frequent in its own class and appears only once in the other class, it is considered as non-discriminent and is filtered while it should not be. Therefore, instead of 0 and 1, the frequency of the motif in the other class is taken into account. In Algorithm 3, for each motif two frequencies related to each class are available, namely f_{EC} and f_{IC}. If S is a

Algorithm 3: Dynamic Support - Feature Minimization

input : $MinSup, N$
output: Frequent Discriminative Feature Subsequences

```
1  begin
2      Mark all proteins as NeedingMotif
3      foreach Class c ∈ {EC, IC} do
4          Len ← 10
5          Set-Of-Subseq ← All subsequences S where length(S) ≥ Len and
           frequency(S) ≥ MinSup
6          foreach Subsequence S ∈ Set-Of-Subseq do
7              foreach Protein p of class c which contains S do
8                  if S can be among N best motifs of p then
9                      p adds S to its set of N-best motifs;
10                 end
11                 if p is expressed by N motifs then
12                     Mark p as Expressed
13                 end
14                 else
15                     Mark p as NeedingMotif
16                 end
17             end
18         end
19         Len ← Len − 1
20         while There are still NeedingMotif proteins ∧ Len > 1 do
21             Set-Of-Subseq ← All subsequences S where length(S) = Len and
               frequency(S) ≥ MinSup
22             go to line 6
23         end
24     end
25 end
```

frequent subsequence of EC class, the confidence of S is defined as the fraction of proteins containing S that belong to EC class:

$$confidence(S) = \frac{f_{EC}}{f_{EC} + f_{IC}} \tag{3.1}$$

and similarly for the motifs of IC class.

When finding motifs of a class, motifs with confidence less than a given threshold can be simply removed. This threshold is called **MinConf** (minimum confidence) and can be inputted as another parameter. If $MinConf$ is set to 100%, frequent motifs appearing only in one class (absolutely discriminative) are discovered.

The only problem that still remains, is the problem of long transactions as we have already explained. However, the extensive efforts in this research demonstrates that it is not wise to put more effort on controlling the length of transactions. Instead, some solutions to increase the ability of the associative classifier for handling long transactions should be explored. These solutions and techniques are elaborated in chapter 4.

3.2 Discriminative and Frequent Partition-Based Subsequences

Previous section concluded with an approach to discover the most discriminative features based on only frequent subsequences or motifs. However, we believe that the presence of a subsequence in special partitions of protein sequences might be more discriminative than the subsequence itself. For example, "*ACDE*" may be a frequent subsequence among both IC and EC proteins, thus is not distinguishing. Nonetheless, "*ACDE*" may appear in the first half of EC protein sequences while in IC proteins it may occur in the second half of the sequences. Here the association of "*ACDE*" and its respective location along proteins is a discriminative pattern. Such a pattern is called "Partition-Based Subsequence", or in short PBS. PBSs are the generalized form of simple subsequences. Simple subsequences are the PBSs whose partition is the whole protein.

Since proteins highly differ in length, the partition should be defined relative to the length (partition-based) i.e.,a protein sequence is divided into 2, 3 or more equal partitions. The presence of frequent subsequences in different partitions is investigated. If protein sequences are assumed to be divided into P partitions, the presence of a subsequence S in the i'th partition of proteins, where $1 \leq i \leq P$, is denoted by $S_{i/P}$. The problem is to find subsequences S with their partitions, i.e.,values for i and P, such that $S_{i/P}$ is frequent and discriminative with respect to $MinSup$ and $MinConf$. Note that i/P in $S_{i/P}$ is not a fraction. For example, $S_{1/2}$ and $S_{2/4}$ are different. The former indicates the presence of S in the first half of proteins while the latter indicates the presence in the second quarter. Figure 3.3 illustrates the difference.

CDE FGH		*KLM NPQ*	
1/2		2/2	
1/4	2/4	3/4	4/4

Figure 3.3: Dividing the virtual protein *CDEFGHKLMNPQ* into 2 and 4 parts and the address of each partition. Trivially location 1/2 and 2/4 are not the same; Neither are 2/2 and 4/4.

In other words, our approach looks at a partition of 100%, then two partitions of 50%, then three partitions of 33% and so on. To explain the algorithm of mining PBSs, a Partition-Frequency Table of a subsequence S should be defined first. In this table, the P'th row is an array of length P. The value in row P and column i indicates $frequency(S_{i/P})$. The first row of this table shows the frequency of subsequence S where each protein is considered as only one sequence (no partitioning). The last row of this table is related to partitioning proteins to a maximum number, namely *MaxPart*, which is given by the user. If *MaxPart* is chosen to be 3, for example, each frequent subsequence possesses a Partition-Frequency Table which is filled as Figure 3.4 illustrates.

After this table is filled with frequencies, the partitions with enough frequency make a frequent PBS. Filling in any slot of this table for all frequent subsequences is a complex task. However, there is no need to fill in the whole table. Indeed, if processed top-down, some partitions can be ignored if their subsuming partition

$frequency(S)$			
$frequency(S_{1/2})$	$frequency(S_{2/2})$		
$frequency(S_{1/3})$	$frequency(S_{2/3})$	$frequency(S_{3/3})$	
$frequency(S_{1/4})$	$frequency(S_{2/4})$	$frequency(S_{3/4})$	$frequency(S_{4/4})$

Figure 3.4: Partition-Frequency table of a subsequence S where partitioning proteins to 1, 2, and 3 is investigated ($MaxPart = 3$)

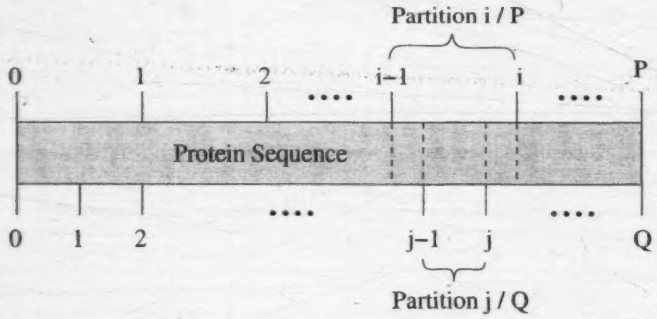


Figure 3.5: Illustration of Equation 3.2

already indicates infrequency. For example, partition 1/2 (first half) encompasses partition 2/4 (second quarter). Therefore, if a subsequence S is not frequent in the partition 1/2, it cannot be frequent in partition 2/4. Assuming that $S_{i/P}$ is not frequent, $S_{j/Q}$ is also infrequent for all smaller partitions j/Q that:

$$Q > P \ And \ \frac{i-1}{P} \leq \frac{j-1}{Q} \ And \ \frac{j}{Q} \leq \frac{i}{P} \tag{3.2}$$

The partition j/Q is totally included in partition i/P and is called a *sub-partition* of i/P. Figure 3.5 illustrates Equation 3.2.

During the filling of the table, after a frequent PBS $S_{i/P}$ of class C is found, its occurrence in the proteins of the other class is counted and then its confidence is computed similar to Equation 3.1. If the confidence is less than a $MinConf$, the PBS is considered non-discriminative and is removed. In case that $S_{i/P}$ reaches the confidence of 100%, there is no need to fill in the sub-partitions of i/P in the Partition-Frequency table of S. Because if j/Q is a sub-partition of i/P, then the confidence of $S_{j/Q}$ is also 100% while its frequecy is less than or equal to $frequency(S_{i/P})$. Therefore, the sub-partitions of i/P are less informative. This

way, partitions are dynamically determined, and *MaxPart* is only an upper bound for the partitions.

Because of the large number of frequent discriminative PBSs, and to reduce the number of features, each protein is restricted to pick only N number of best PBSs that match with it. If a PBS is not selected by any protein, it is removed. For selecting its N best features, a protein ranks its PBSs based on different metrics. In our approach, confidence, length and frequency are respectively the primary, secondary and final ranking metric. For example, between two PBSs with equal confidence, the longer one has a higher rank. Other metrics and priorities can be set by the user depending on the importance of feature properties.

Algorithm 4 is a summary of what was discussed. If *MaxPart* is set to 1, i.e.,proteins are considered as only one partition of 100%, the algorithm works similar to Algorithm 3, explained in the previous section and only frequent subsequences regardless of partition are mined. However, there is a small difference in the selection of N-best motifs. In Algorithm 3, the longer a protein is, the more important it is to be selected by proteins. But in Algorithm 4, the priority is with the most confident motifs as explained above. Experimentally, Algorithm 4 finds better feature motifs in the case of $maxPart = 1$ than Algorithm 3.

As an advantage to our previous algorithms, this algorithm considers that expressing test proteins is more important than expressing training proteins. If a test protein remains silent in the resulted feature space, no rule can classify that protein because rules are made up of features. We observed that some good features are mined that can express a test protein P, but they are not among the N-best features of the training proteins. Moreover, none of the N-best features of the training proteins express P. In such frequent cases, P remains silent if the N-best features of only the training proteins build the feature space. Hence, each frequent and discriminative PBS, which is mined only based on the training proteins, is also given to the test proteins matching with it. A test protein keeps that PBS if the PBS is among the N-best features of the protein. Line 20 of Algorithm 4 is where this happens.

When proteins select their discriminative and frequent features, a union of PBSs of all the training (EC and IC class) and unlabelled test proteins is made, and then

Algorithm 4: Mining frequent and discriminative PBSs

input : $MinSup, MinConf, MaxPart, N$

```
// MaxPart is the desired maximum number of
partitions
// Each protein is to be expressed by at most N
motifs
```

output: Frequent Discriminative PBSs

begin
  foreach *Class* $c \in \{EC, IC\}$ **do**
    Set-Of-Subseq ← All subsequences S in class c with $frequency \geq MinSup$
    foreach *Subsequence* $S \in$ *Set-Of-Subseq* **do**
      Create Partition-Frequency table of S; (partition proteins up to *MaxPart* partitions)
      `//The formula 3.2 is used to detect non-frequent partitions beforehand`
      foreach *Frequent Partition* i/P *in Partition-Frequency table* **do**
        Count $frequency(S_{i/P})$ in the other class;
        Compute $confidence(S_{i/P})$; `//Use equation 3.1`
        if $confidence(S_{i/P}) < MinConf$ **then**
          `//It is not discriminative enough`
          Remove $S_{i/P}$;
        end
      end
    end
    `//At this point all frequent discriminative PBSs of class c are available`
    foreach *PBS* $S_{i/P}$ *(just mined)* **do**
      foreach *Protein* $p \in \{classc \cup testdata\}$ *which contains* S *in its* i/P *partition* **do**
        if $S_{i/P}$ *can be among N best motifs of* p **then**
          p adds $S_{i/P}$ to its set of N-best motifs
        end
      end
    end
  end
end

proteins are represented by the PBSs from the union set with which they can match. The feature dataset will be in the form of a transactional dataset. Afterwards, test proteins that are still silent request for *reconsideration*. In the reconsideration process, a silent protein gets expressed by the PBSs from the union set that it can *partially match*. Test protein T is said to *partially match* with $S_{i/P}$ if it cannot match with this PBS but there exists a subsequence S' such that T and $S'_{i/P}$ can match and $EditDistance(S, S') = 1$. *Edit distance* [11] is an appropriate metric for measuring the amount of difference between two strings. Edit distance of 1 implies that S' is made by inserting a character to or deleting a character from S or by substituting a character in S with another character. For example, if $S = ABCD$, S' is $ABFCD$, ABD, $AB?D$ or ... where '?' is a don't-care. More explanation about edit distance is given in Section 4.2. According to our experiments, reconsideration reduces the number of silent proteins by more then 10%.

The reason we chose the edit distance of 1, and not more, is that frequent subsequences are not very long. Roughly, their length varies from 3 to 10 in general. With the above notation, allowing more edit distances may lead to subsequences S' that are no longer similar to the original subsequence S. More importanly, reconsideration is an expensive task and becomes more complex if the higher edit distances are allowed. Our current approach for reconsideration includes creating all subsequences S', for all PBSs $S_{i/P}$, and then checking all silent proteins if they can match any S' in their i/P partition. As the length of S or the number of silent proteins increase, more comparisons should be made.

Chapter 4

Associative Classification for Protein Localization

As the previous sections explained, the output of the feature extraction phase is a transactional feature dataset. Different algorithms can be used to learn protein localizations and classify unseen proteins to EC or IC localizations. However, a classifier with interpretable output model is prefered. Moreover, if the accuracy of such a classifier is high, two worthwhile goals are achieved: First, an accurate classification model is found to predict the location of new unknown proteins. Second, it can be inferred that a good selection of discriminative and descriptive features of EC and IC proteins have been discovered. Although each transactional dataset can be converted into relational format, it is better for the classifier to work with transactional data since our feature datasets are originally transactional. If those feature datasets are transformed to relational format, all the attributes become binary (presence or absence of features). If such a dataset is n-dimensional, each protein becomes a corner of the n-dimensional unit hyper-cube in the feature space. Such data is very unlikely to be linearly or even easily separable and many classification algorithms may fail.

Among different learning methods, associative classification is a good algorithm that learns from transactional data. Beyond its ability to generate confident and interpretable rules, the associative classifier has another advantage. Unlike many classifiers such as artificial neural networks [24] or SVM [27], it does not need delicate or complicated parameter settings. It takes in a few tangible parameters

$MinSup$ (minimum support of rules), $MinConf$ (minimum confidence of rules) and optionaly, the length of rules ($MinLen$ and $MaxLen$).

An associative classifier [22, 26] integrates methods for association rule mining and classification. The input is a transactional dataset and the output is a set of frequent and confident associative rules of the form $X \Rightarrow C$, where X is a frequent itemset (in our case, a set of motifs or PBSs) and C is a cell location. Thus, finding classification rules for a class C includes discovering frequent itemsets X with a support greater than a threshold ($MinSup$), and then pruning rules based on a confidence threshold ($MinConf$) and some other criteria. Support of a rule $X \Rightarrow C$ is the fraction of proteins from class C that can match X. The confidence of this rule is similar to Equation 3.1.

Localization rules can be of different lengths. The length of a rule is reffered to the size of feature set X, i.e.,predicate of the rule. Therefore, each subsequence-based motif by its own is a rule of size 1. For example if "$KLMN_{1/2}$" is a frequent PBS of EC proteins with 80% confidence (with the definition of confidence for motifs), it produces the rule "$KLMN_{1/2}$" $\Rightarrow EC, conf = 80\%$. However, the reason we use associative classifier on the feature dataset is to find more confident rules. The frequent associations of items can generate longer rules with higher confidence than that of each individual feature.

4.1 Building Associative Rule Classifier (Training Phase)

In association rule mining, discovering frequent itemsets is a preliminary task whose processing complexity depends on the length of transactions and the number of items. As transactions get longer while the number of items in that dataset is not large, items and possibly their different associations repeat in many transactions. Hence, many frequent itemsets of different lengths may be mined. In these cases, mining itemsets takes very long time and the itemsets may not be stored or managed in a limited memory. Therefore, the classification algorithm should take serious care of memory management in the case of long transactions.

During our study, we obtained many feature datasets with long transactions. For

example, in one of the average size feature datasets of our experiments, the transactions representing proteins averaged a length of 55. Almost 10% of the transactions had a length between 350 and 550 PBSs, which is remarkably long, and almost 88% are represented by transactions of length around 25. The histogram of transaction lengths of this feature dataset is shown in Figure 4.1. In such situations, so many frequent itemsets (potential rules) are mined that the classification algorithm has to consider effective means of selecting appropriate rules. Moreover, before rule pruning, excessive memory is required.

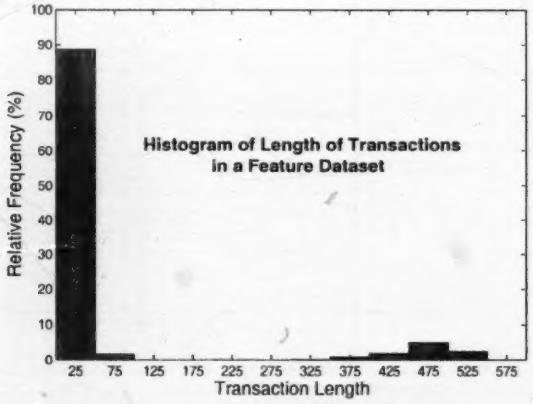


Figure 4.1: The histogram of the length of transactions in our best feature dataset on which we coud make the most accurat classifier

A discovered frequent itemset X from a class C directly corresponds to the rule $X \Rightarrow C$. As explained in the Algorithm 5, each frequent itemset is potentially abridged, then the rule confidence is used to prune those rules that are less confident than $MinConf$. Other pruning strategies can be applied too. Each of the mentioned steps in mining proper rules is explained in the following sections.

4.1.1 Mining Frequent Itemsets

To mine frequent itemsets, we first tried one of the fastest and most efficient implementations of association rule mining by Borgelt [7]. Nonetheless, since the Apriori algorithm [30] is based on a breadth-first search of the itemset lattice, it needs much memory. All nodes of search tree at a level n, which are length-n itemsets, need to

be stored in main memory to be expanded later in the next level to generate length $n+1$ itemsets. As the length of itemsets increases, the amount of required memory increases almost exponentially until the itemsets cease to grow due to their large length and lack of frequency. In our case, the Apriori algorithm lacked memory[1] and failed in the middle of mining itemsets of length more than 5.

An alternative to the Apriori algorithm is Eclat [25], efficiently implemented by Borgelt [7]. Eclat mines frequent itemsets by the depth-first search of the itemset lattice. The efficient Eclat code by Borgelt, which is used in our research, utilizes a bitmap vertical layout of the transactions, i.e.,a matrix in which each row is related to an item and each column is related to a transaction ID. Ones and zeros in each row and column intersection indicate whether or not the item of the row exists in transaction of the related columns. Figure 4.2 shows an example of this layout. In this layout, the frequency of each itemset is simply computed by "and"ing transaction lists. For example, the output of "and" operation on the binary list of B (0110) and E (0011) is 0010 which expresses the absence of itemset {B, E} in transactions 1, 2 and 4, and its presence in transaction 3.

Transaction ID	Items
#1	A, C, D
#2	A, B
#3	B, D, E
#4	A, E

(a) Normal Layout

Item	Transaction IDs			
	#1	#2	#3	#4
A	1	1	0	1
B	0	1	1	0
C	1	0	0	0
D	1	0	1	0
E	0	0	1	1

(b) Bitmap Vertical Layout

Figure 4.2: Two layouts of storing transactions. The bitmap vertical layout is used in Eclat

4.1.2 Abridging Itemsets

Itemsets could be redundant and abridging some itemsets can be helpful. Abridging consists of eliminating from an itemset any item that is already represented and implied by another item in the itemset. In our context, items are features, i.e.,PBSs.

[1]On a public local machine, in the Department of Computing Science, University of Alberta, with 8GB main memroy

Algorithm 5: Building of Associative Rule Classifier

input : $minSup$, $minConf$, $minLen$, $maxLen$
output: Set of localization rules

```
1  begin
2      DataSet_EC ← All EC proteins from feature dataset
3      DataSet_IC ← All IC proteins from feature dataset
4      foreach class c ∈ {EC, IC} do
5          RuleSet_c ← {}
6          //Rules that imply class c will be stored in
           RuleSet_c
7          while Eclat finds next Frequent Itemset X with parameters minSup,
           minLen, maxLen do
8              X ← Simplify the itemset X
9              if (X ⇒ c) ∉ RuleSet_c then
10                 Compute frequency_o(X): frequency of X in the Other
                   DataSet
11                 Compute Conf(X ⇒ c) : frequency_c(X) / (frequency_c(X)+frequency_o(X))
12                 if Conf(X ⇒ c) < minConf then
13                     Prune the rule.
14                 end
15                 else
16                     Try other pruning techniques.
17                     if Not pruned then
18                         Add (X ⇒ c) with its confidence to RuleSet_c
19                     end
20                 end
21             end
22         end
23     end
24 end
```

F_1 is a subfeature of F_2 (F_2 is called super-feature) and is written $F_1 \preceq F_2$ if and only if all the proteins that match F_1, also match F_2. For example, "$JKLM$"$_{1/4} \preceq$ "KL"$_{1/2}$: a protein containing "$JKLM$" in its first quarter has trivially contained "KL" in the first half.

The definition of sub-feature is as follows:

$T_{j/Q} \preceq S_{i/P} \Leftrightarrow S$ is a subsequence of T, and partition i/P surrounds partition j/Q, i.e.,(refer to Equation 3.2, previous chapter)

$$Q \geq P \ And \ \frac{i-1}{P} \leq \frac{j-1}{Q} \ And \ \frac{j}{Q} \leq \frac{i}{P}$$

Therefore, if the predicate of a rule contains two motifs M_1 and M_2 where $M_1 \preceq M_2$, the rule is abridged by removing M_2. For example
"KL"$_{1/2}$, "$JKLM$"$_{1/4} \Rightarrow EC$ is simplified to "$JKLM$"$_{1/4} \Rightarrow EC$.

Abridging should be done iteratively to the rule until no pair of motifs or PBSs in the rule is found with one the sub-feature of another. For example, the 4-itemset {"KL", "KLM", "EF", "$CDEF$"} should be abridged to the 2-itemset {"KLM", "$CDEF$"}. Line 8 of Algorithm 5 runs this process. Abridging decreases the number of rules dramaticly since there are many rules that can be simplified to a specific rule, and only one copy of that short rule is stored.

Given two motifs N and M, learning whether N is a sub-feature of M is linear in terms of the length of motifs. However, this linear comparison can be faster (in constant time) using hash functions. To do so, after all features are mined, each motif is mapped to the set of all its sub-features using a hash map. Moreover, to store the set of sub-features of a motif, a hash set is used. Therefore in the hash map, keys are motifs and values are the hash sets. To compare whether $N \preceq M$, M is searched in the hash map and its hash set is accessed in constant time. Then the availability of N in the retrieved hash set is checked in $O(1)$. Therefore, this data structure helps to check the sub-feature comparison in constant time. This strategy speeds up the execution when hundreds of thousands of raw frequent itemsets are generated that need to be abridged and filtered.

4.1.3 Computing the Confidence of a Rule

As we explained earlier, the confidence of $X \Rightarrow C$, where X is an itemset, depends on the frequency of X in both classes. The frequency in class C is available as soon as X is mined as a frequent itemset of class C. The important issue is counting its frequency in the other class. For fast and efficient computation of this frequency, the following approach is used:

1. All the features, e.g.,frequent subsequences or PBSs, should be assigned numerical IDs. Then instead of representing a protein by its features, the protein is represented by the IDs of its features. In this way, the feature dataset looks like transactions of numerical items.

2. Items (i.e.,feature IDs) in each transaction (i.e.,protein) are sorted in increasing order.

3. Transactions of each class are inserted into two Trie structures, namely $Trie\{EC\}$ and $Trie\{IC\}$. As we explained in Section 3.1.6, a "trie" is a tree structure in which the internal nodes of each level are sorted. The main property of trie is its fast search feasibility. In our case, the internal nodes of the trie store the items. The direct path from the root to a leaf node is equivalent to an itemset. For computational efficiency, the number of leaves of the sub-trie rooted at a node m is also stored in node m.

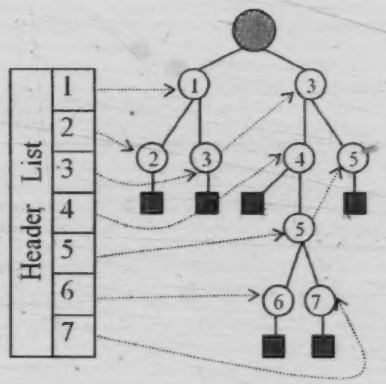
4. Whenever an itemset X of class C is generated, we sort X in the increasing order of its items.

5. Find all the nodes N of $Trie\{other\ class\}$ that match the first item in X. Rooted at nodes N, traverse (Depth-First) the sub-tries to find the matches with X. Whenever X matches the trie at a node m, the algorithm counts the number of leaves of the sub-trie rooted at node m (which is stored in node m) and stops traversing deeper down the node m, and tries matching X with other branches. For fast finding of Nodes N, which match the first item of X, we use a list, called header list, which contains all the items in the trie. By

following the pointers from an item I in the header list, we can find all the occurrences (matches) of item I in the trie. Making these pointers is simply done when the trie is being constructed.

Localization	Transaction
IC	1, 2
IC	1, 3
IC	3, 4
IC	3, 4, 5, 6
IC	3, 4, 5, 7
IC	3, 5

(a) Feature dataset of IC proteins



(b) $Trie\{IC\}$

Figure 4.3: The Trie representation of IC protein transactions

Figure 4.3 shows how a trie represents IC transactions ($Trie\{IC\}$). Suppose $X = \{3, 5\}$ is a frequent itemset of EC proteins. To obtain the frequency of X among IC proteins, the two nodes of $Trie\{IC\}$ containing the value 3 are identified first. Of those two nodes, the leftmost one cannot make a match, but the other node can make two matches at the nodes containing value 5. One of those two nodes with value of 5 has 2 leaves in its downward sub-trie and the other one has just 1 leaf. Therefore, the total occurrence of X in $Trie\{IC\}$ is three, and now the confidence of $\{3, 5\} \Rightarrow EC$ can be easily computed.

4.1.4 Pruning the Rules

The minimum confidence requirement ($minConf$) prunes many rules and lets only confident rules remain. However, the number of confident rules is still large and some other pruning techniques are required, as line 16 of Algorithm 5 suggests. These techniques are as follows:

1. If the confidence of a rule, $X \Rightarrow c$, reaches 100%, any expansion of its predicate results in a rule with 100% confidence too, i.e.,$X \cup Y \Rightarrow c, conf = 100\%$,

where X and Y are two disjoint itemsets and c is a class label. The reason is clear: all proteins matching the second rule, can also match the first rule, and the first rule 100% guarantees them to be in class c. In this case, keeping the first rule suffices, and any other expanded rule is not useful. This technique prunes many rules especially in Eclat with the depth-first search of the itemset lattice, because expansion of a rule falls in the deeper levels of lattice, and Eclat can stop going deeper in the recursion path as soon as it finds a 100% confident rule.

2. If R is a rule with confidence $conf$, all the sub-rules of R with confidence less than $conf$ should be pruned. R_1 is a sub-rule of R_2 (R_2 is called super-rule) and is written $R_1 \sqsubset R_2$ if and only if any protein that matches R_1 can also match R_2 (i.e.,R_2 is more general), further, R_1 and R_2 should imply the same class. In other words if R_1 is:

$$n_1, n_2, \ldots n_i \Rightarrow C \ \ with\ conf = \alpha$$

and R_2 is:

$$m_1, m_2, \ldots m_j \Rightarrow C \ \ with\ conf = \beta$$

Then $R_1 \sqsubseteq R_2$ if and only if:

   (a) $i \geq j$, i.e.,the length of a sub-rule can not be less than that of its super-rule.

   (b) For each item m_b ($1 \leq b \leq j$), there must be an item n_a ($1 \leq a \leq i$) such that $n_a \preceq m_b$. i.e.,at least one sub-feature of each m_b must be found in the sub-rule.

If $\alpha \leq \beta$ then R_2 is much worth keeping as a more general rule than R_1, and R_1 should be removed. In this case, R_1 is called the removable sub-rule of R_2. For example suppose R_1 is:

$$\text{“}JKLM\text{”}_{2/4}, \text{“}PQRST\text{”}_{4/5} \Rightarrow EC \ \ with\ conf = 60\%$$

and R_2 is:

$$\text{“}KL\text{”}_{1/2} \Rightarrow EC \ \ with\ conf = 90\%$$

R_1 should be removed and R_2 kept because "$JKLM$"$_{2/4} \leq$ "KL"$_{1/2}$.

When a new rule is to be added in a rule set, this rule has to be compared to all the older rules. Any older rule that is a removable sub-rule of the new rule, is removed. If the new rule is a removable sub-rule of any older rule then it is not added to the set. The data structure used for this rule set is also a Trie similar to Figure 4.3.

4.2 Evaluating Associative Rule Classifier (Testing Phase)

Given an unknown protein P, a rule can match P when the antecedent of the rule applies for the features representing P. The rules that match P localize the protein as EC or IC. To decide between the two classes, there are different possibilities. One option is to find the rule with the highest confidence, and the predicted class of the test protein is the class of that rule. There is a drawback to this selection: the effect of other rules is ignored. For example consider Figure 4.4. Although the first rule indicates that 90% of proteins that contain feature 1 are IC, the association of 1 with 2, 3, 4 or 5 is something that happens in EC proteins in more than 80% of the cases (based on the EC rules), which is exactly what is seen in the test protein. Hence, it is more reasonable to classify the protein as EC rather than IC. Nonetheless, with the most confident rule, a different classification is made. As an alternative, the average confidence of the matching rules of each class can be considered. The class with the highest average of confidences is assigned to P. There is an exceptional case for which confidence averaging is not used. Whenever a rule with 100% confidence matches the test protein P, the class label of that rule is assigned to P as long as there is no other 100% confident rule of the other class. The reason is that 100% confident rules exhibit unique facts (derived from the training samples) about proteins of a class that is never met in the proteins of the other class.

In a few cases, a test protein cannot match any rule from any class. Moreover, there are cases that a test protein is equally classified as both EC and IC. The latter happens mainly when there are EC and IC rules with 100% confidence, and both can match and classify the test protein, or when the confidence averages are the

Test Protein: $\{1, 2, 3, 4, 5\}, class = ?$

Matching Rule	Confidence
$1 \Rightarrow IC$	**90%**
$2, 3 \Rightarrow IC$	71%
$3, 4 \Rightarrow IC$	68%
$1, 2, 3 \Rightarrow EC$	85%
$1, 2, 4 \Rightarrow EC$	88%
$1, 5 \Rightarrow EC$	86%

Figure 4.4: An example of a test protein and the localization rules matching the protein

same. It should be noticed that the confidence of 100% of a rule is obtained only based on the training data. Hence, it is possible that a test protein of the opposite class, which is not seen before, contradicts such a rule. We call such test proteins *Undecided*. In order to determine a label for undecided proteins, there are two strategies:

1. Undecided proteins are de-facto classified as IC, the majority class. This is the simplest decision.

2. Undecided proteins are classified by another classifier. This classifier is called *secondary* and associative classifier is called *primary*.

In this work, we use Nearest Neighbor (NN) classifier as the secondary predictor for classifying only around 20% of the proteins that are undecided. NN is a simple algorithm with no input parameter, and is in accord with our goal to achieve an interpretable model because its classification is nothing more than detecting proteins with similar sequences.

In NN classifier, the distance of an unknown test protein P to all of the labelled proteins of the training data is computed. The class label of the closest protein is assigned to P. *Edit* or *Levenshtein distance* [11] is the distance measure used by our NN classifier. Edit distance for measuring the difference of two strings has applications in structural or functional studies of biological sequences, textual database retrieval, spelling correction algorithms, etc . This distance is defined as the minimum number of edit operations required to transform one string to the other. The

permitted operations are insertion, deletion, or substitution of a single character. Edit distance for the difference of two protein sequences is a more realistic metric as the mentioned operations are equivalent to mutations that happen through biochemical processes. Figure 4.5 partially shows two extracellular protein sequences found in radish. They are very similar and seem to be originated from a same sequence but with a few mutations in their composition:

MAKF(A)SI(VA)LLF(A)ALV(V)FAAFEAPT(V)VEA(-)KLCERSSGTWSGVCGNNN . .

MAKF(V)SI(IT)LLF(V)ALV(L)FAAFEAPT(M)VEA(Q)KLCERSSGTWSGVCGNNN . .

Figure 4.5: Two similar extracellular proteins that are mutated from a same sequence.

In Chapter 5, it is demonstrated that the second strategy (i.e.,NN) works better than the first one (i.e.,majority class).

One might ask why not using NN classifier overally instead of having it as the secondary classifier? The answer is that computation of edit distance is an expensive task with the time complexity of $O(mn)$ where m and n are the length of input sequences [11]. When sequences are proteins, m and n are very large and edit distance computation takes much time. To depict this cost, the construction of edit distance matrix for the 3149 proteins of our dataset took more than 2 days on a very strong machine with the following specifications:

- dual CPUs - Quad core (for 8 processors)
- 32 GB of memory

Our experiments show that the primary classifier, i.e.,associative classifier on the PBS-based feature dataset, classifies 79% of the test data in a short time. Therefore, the need for using the secondary classifier remains for only 21% of the data and the total classification is done in a reasonable time.

Even if we consider no limit in computation time, using NN as the overall classifier is not a good alternative. Experiments demonstrate that the combination of the primary and secondary classifiers results in a more accurate model with almost 6% rise in the F-Measure than using NN alone.

Chapter 5

Experimental Results

In this chapter, we evaluate our models for predicting subcellular localization and demonstrate the discriminative power of partition-based subsequences. The main classification algorithm that has been the focus of this work is the associative classifier. However, because of the growing interest in SVM and its strong ability to classify high dimensional data, we compare our results with those of SVM. Decision tree classifiers, generating a human readable and interpretable model, are also studied.

5.1 Dataset and Evaluation Methodology

We performed our method on a plant protein dataset from the Proteome Analyst Project [46] at the University of Alberta. The dataset is constructed from SWISS-PROT. After cleaning the data, i.e.,removing repetitive or defective proteins which contain nonexistent amino acids, 3,149 proteins remained. The portion of EC proteins is only 4% of the data which shows the severe imbalance in the data.

To evaluate the performance of classifiers, *Overall Accuracy* is often used. However, this is usually inappropriate particularly with imbalanced data. In our case with 96% of proteins being IC, a classifier that always classifies as IC achieves the overall accuracy of 96% while no EC proteins are correctly classified. Instead, we chose precision, recall and F-measure with respect to EC (i.e.,the target class). These three measures are commonly used in this field of research. Using them in our work allows easier comparison with the related approaches. We did not choose

graphical measures such as cost curves [10] because they are more complex and dependent on the misclassification costs.

Based on the confusion matrix shown in table 5.1, Precision(P), Recall(R) and F-Measure (a harmonic average of precision and recall) of EC prediction are defined as:

$$P = \frac{TP}{TP + FP}, \quad R = \frac{TP}{TP + FN}, \quad FMeasure = \frac{2PR}{P + R}$$

	Actually EC	Actually IC
Predicted as EC	TP	FP
Predicted as IC	FN	TN

Table 5.1: Confusion Matrix

To have a more reliable evaluation, all the feature extraction and classification experiments are based on a 3-fold cross validation. The dataset is initially shuffled and divided into three equal parts (folds) such that the distribution of EC and IC proteins in the three folds agree. Each run takes two folds for training and the other fold for testing. After the features are extracted from the training proteins, both test and training proteins are represented by the set of mined features. Then, by training a classifier on the training data, the prediction model is evaluated on the test data. In the end, the F-Measures from each of the three runs are averaged as the EC prediction accuracy. To have a fair comparison, exactly the same folds are used in all the experiments, also with SVM and decision trees.

5.2 Mining Frequent Partition-Based Subsequences

Mining frequent PBSs depends on the parameters N, $MinConf$, $MinSup$, $MaxPart$ and $MinLen$. A proper setting for these parameters should prevent *silent* proteins, i.e.,proteins not expressed by any of the mined features. Test proteins are more important not to be silent than training proteins. If a test protein is silent, it can never be classified by any rule, and becomes the zero vector $\vec{0}$ in the n-dimensional feature space.

The following shows the setting of the parameters:

- $N = 1$: Each protein selects its top best PBS. Larger values of N result in a longer transaction in the feature dataset and make classification harder.
- $MinConf = 50\%$: If the confidence of a PBS is less than 50%, it is useless because instead of being a frequent pattern in its own class, it is more frequent in the other class.
- $MaxPart = 10$: $MaxPart$ is initially set to 10, which means considering a protein as a partition of 100%, 2 halves, 3 thirds, ... and 10 partitions of 10%. Further experiments are done in Section 5.4 to show the effect of partitioning on the prediction.

The setting of $MinLen$ and $MinSup$ is more difficult because they are the most sensitive parameters. The longer a motif, the more discriminant but less frequent. For example, assume $s_1 = ACD$ and $s_2 = ACDEFGHJK$ are frequent subsequences of a class C. A random protein sequence is more probable to contain s_1 than s_2, thus s_1 is expected to be more occurring in the other class than s_2. Similarly, the frequency of s_1 in class C is likely to be higher than that of s_2. Hence, longer motifs are generally more confident. They also convey more information and are preferred. On the other hand, silent proteins cannot be completely avoided unless $MinLen$ is set to 4 (Figure 5.1).

About $MinSup$, the larger it is, the more frequent and meaningful patterns are discovered but more silent proteins are observed in that not all proteins have frequent features. In short, a proper setting for $MinSup$ and $MinLen$ should result in longer motifs and less number of silent proteins.

We considered the values 0.2%, 0.5%, 1 to 5% for $MinSup$, and values 4 to 8 for $MinLen$. As we explain in this section, a combination of these settings to generate high quality PBSs is investigated. Note that the length of motifs is forced to be less than 100 and the minimum frequency of a motif is not less than 2, i.e.,if motifs of a class C with N proteins is to be mined, then $MinFrequency = Max(2, MinSup*N)$.

For a certain $MinLen$, with lower minimum supports, more features are generated and less silent proteins are seen. Moreover, longer motifs find the chance to

show up since their frequency (support) is generally less. Figure 5.1 and Figure 5.2 show the influence of $MinSup$ on the number of silent proteins and the length of mined motifs. The $MinSup$ of 0.2% seems the best at which less silent proteins and longer motifs are observed.

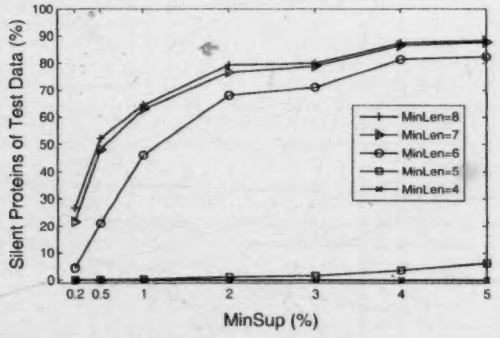


Figure 5.1: The influence of $MinSup$ on the number of silent proteins of test data

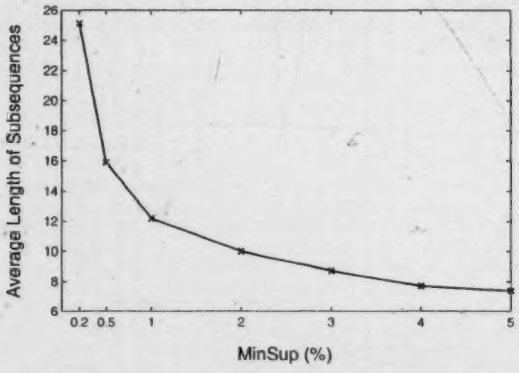


Figure 5.2: Average length of subsequences for different $MinSup$ values where $MinLen = 3$

The support of 0.2% is considered low and if $MinLen$ is going to be small too, then the features will not be discriminative enough. The reason is that although the PBSs are around 100% confident, the confidences are measured only based on the training data, and for shorter subsequences it is likely to occur in some previously-

unseen test protein of the opposite class, while it is not the case for long motifs. Thus, at $MinSup$ of 0.2%, higher $MinLen$ should be chosen.

Table 5.2 demonstrates that increasing $MinLen$ has an influence on decreasing *potentially undecided test proteins*. A test protein is called *potentially undecided* if it is represented by at least two features, one from EC and one from IC class. Later in the classification, such a protein is possible to be classified as both EC and IC, and becomes undecided. With the hidden labels of test proteins, this measure is a good indicator of how discriminative the features are when they come to the scope of test data. According to Table 5.2, a minimum length of 7 and 8 prevents from potentially undecided proteins. Between these two lengths, $MinLen = 7$ is selected, for which less silent proteins are observed (refer to Figure 5.1).

MinSup = 0.2%	MinLen				
	4	5	6	7	8
Potentially Undecided Proteins of Test Data	6.35 %	1.08 %	0.51 %	0.06 %	0.00 %

Table 5.2: The decrease of potentially undecided test proteins when $MinLen$ increases

This selection of parameter values is feasible without the need to know the labels of test proteins. Later in the next section, when the class labels of test proteins are uncovered, we experimentally prove that no other setting than $MinSup = 0.2\%$ and $MinLen = 7$ can lead to a higher accuracy (F-Measure). With these parameter values, on average, 864 PBSs are mined only 39 of which are for EC class.

5.3 Classification Algorithms and the Prediction Model Evaluation

A combined model of associative classifier and first nearest neighbor (1NN) classifier creates our main model. To have an understanding of how well our proposed algorithm works, the results of the following classifiers on the same data are compared with our model:

- Associative classifier, where undecided proteins are classified as IC,
- 1NN classifier,

- SVM classifier,
- Decision Tree,
- Combination of SVM and 1NN,
- Combination of decision tree and 1NN.

5.3.1 1NN Classifier

1NN is known as lazy classifier which needs no training. With edit distance as the distance measure, each test protein finds the closest protein sequence of training data. The localization label of that protein is chosen to localize the test protein. The result of this parameterless classifier is as follows:

Average Recall (%)	Average Precision (%)	Average F-Measure (%)
88.22	87.58	**87.83**

Table 5.3: Evaluation of 1NN classifier.

As discussed in Section 4.2, this classifier solely is computationally expensive. The run time of this classifier, i.e.,the computation of the edit distance matrix of the proteins, took almost 50 hours.

5.3.2 Associative Classifier

Associative classifier should first mine frequent itemsets from the transactional feature dataset. For this mining, an efficient, publicly available implementations of Eclat algorithm [7] is used. Eclat uses a depth-first traversal of the itemset lattice. After rules are generated and classification begins, undecided proteins are classified as IC, which is the major class.

Generally, the accuracy of the associative classifier depends on parameters $MinSup^*$ and $MinConf^*$. The star over the name of these parameters is to distinguish them from the $MinSup$ and $MinConf$ used for feature selection. In our case where PBSs are highly confident (around 100%), very confident rules (around 100%) are generated from the PBSs. Thus $MinConf^*$ seems not affecting the prediction accuracy. In our experiments, the different $MinConf^*$ settings of 50%, 70% and 90%

$50\% \leq$ MinConf* $\leq 90\%$						
MinSup* (%)	0.2	1	2	3	4	5
Undecided (Classified as IC) (%)	**21.76**	72.35	81.33	84.09	87.33	89.08
F-Measure (%)	**80.04**	80.28	80.28	80.28	79.24	66.68

Table 5.4: F-Measure and the rate of undecided proteins in single associative classifier.

have been tried but the same results have been obtained. Table 5.4 compares the F-Measures at different supports.

Based on Table 5.4, the best F-Measure, 80.04%, is achieved when $MinSup^* = 0.2\%$, i.e.,the same minimum support that is already used for feature extraction. With this support, relatively the least portion of proteins, 21.76%, become undecided. Table 5.5 summarizes the best result in which recall is not satisfactory because all the undecided EC test proteins are de facto classified as IC.

Average Recall (%)	Average Precision (%)	Average F-Measure (%)
70.04	93.72	80.04

Table 5.5: Summary of the best result from associative classifier.

5.3.3 SVM Classifier

With data represented as vectors in the multi-dimensional feature space, SVM [27] finds the hyperplane that best separates instances of two classes. The hyperplane divides the feature space into two sub-spaces each for one class. Unknown data is simply classified based on the sub-space it is located in. For the datasets that are not linearly separable, SVM makes use of kernel functions or soft margin separation hyperplanes.

To use SVM, our feature dataset, which obtained the best result with our approach, is transformed from transactional to a relational dataset with a fixed dimensionality. This is simply done by creating a matrix in which each column represents a PBS, and each row represents a protein as a binary vector. Trivially silent proteins are all represented as the zero vector $\vec{0}$ in this space. The dimensionality of the feature dataset, is equal to the total number of PBSs, which is 864. Such a dataset is considered very high dimensional. However, SVM can handle high dimensionality

well.

We used LIBSVM, an available implementation of SVM [8]. In SVM, there are two important parameters to be set: the kernel function and the parameter C (Cost) [36]. Table 5.6 summerizes the F-Measures of SVM classifiers obtained from different kernel functions and costs[1]. The parameter gamma (γ) for the Radial Basis Function, and the scale factor of Sigmoid Kernel is suggested by LIBSVM to be 1/k, where k is the dimensionality of data. Note that polynomial kernel function (degrees 2 to 5) was also tried but the resulted SVM model never learnt EC proteins (F-Measure = 0).

As Table 5.6 shows, different costs have slight effects on F-Measure when linear kernel function is used. Linear kernel beats the other kernel functions with an F-Measure of 72.93% when cost is set to 100. The result of this model is shown in Table 5.7

	F-Measure		
	Linear Kernel	**Sigmoid Kernel**	**Radial Basis Function kernel**
C = 1	72.62	0	0
C = 10	72.76	0	0
C = 100	**72.93**	15.71	34.06
C = 1000	72.93	72.76	71.56
C = 10000	72.93	72.76	71.56

Table 5.6: SVM Classification using different Kernels

Average Recall (%)	**Average Precision (%)**	**Average F-Measure (%)**
59.87	93.65	72.93

Table 5.7: Summary of the best result from SVM

5.3.4 Decision Tree

Decision tree as a classifier is a flow-chart-like tree structure in which each internal node denotes a test on an attribute, a branch is an outcome of the test, and the leaf

[1]Because of the similarity of our problem to what She et al. [36] has done, we used the Cost values they have selected in their experiments

nodes are class labels (classification decision) or class distributions. To classify a data sample, internal nodes, starting from the root, make tests on the data and pass the decision along the branches until a leaf is reached with a decision on the class label. ID3, proposed by Quinlan [34] in 1986, is a basic algorithm to generate decision tree. C4.5 is an extension of ID3 by the same author [35]. Handling continuous attributes, handling missing values, and pruning trees after creation are some of the improvements C4.5 has made.

The reason why decision tree is selected to be studied is that the high discriminative power of PBSs, with confidences around 100%, makes them good internal node tests for clear decisions. However, experiments show that the associative classifier is not outperformed by decision tree approaches. Weka [41], an open source data mining package, has implemented ID3 and C4.5. Table 5.8 shows the result of classification using these two algorithms. ID3, with the 72.78% F-Measure, works better than C4.5 in this case.

Algorithm	Recall (%)	Precision (%)	F-Measure (%)
ID3	59.07	94.87	**72.79**
C4.5	53.57	97.33	68.99

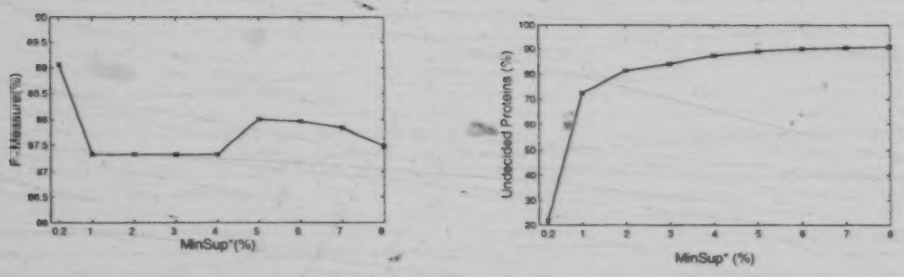
Table 5.8: The result of ID3 and C4.5, two decision-tree-based classifiers.

5.3.5 Combination of Associative and 1NN Classifiers

To construct this combined classifier, an associative classifier is applied to a protein with unknown localization. If the protein is undecided[2], 1NN classifier decides on the class label. Figure 5.3 shows the F-Measure of this classifier at different minimum supports. As we mentioned earlier, in our experiments F-Measure varies only by $MinSup^*$. For a constant $MinSup^*$, F-Measure of the model with $MinConf^*$ set to 50%, 70% and 90% does not vary.

Generally when $MinSup^*$ increases, the rules composed of low support PBSs cannot be discovered, and the proteins expressed by those PBSs cannot match the frequent rules and become undecided. According to Table 5.4, with the minimum supports other than 0.2% more than 72% of the proteins become undecided and

[2]refer to Section 4.2 for the definition of undecided protein



(a) (b)

Figure 5.3: 5.3(a) The F-Measure of the combined model of associative and 1NN classifiers, and 5.3(b) The portion of undecided proteins that are localized by 1NN classifier.

should be predicted by 1NN classifier, while 1NN is expected to be a helper for associative classifier not a classifier to predict most of the test data. When $MinSup^*$ is set to 0.2%, only 21% of the data is classified by the secondary classifier. Moreover, the highest F-Measure (89.06%) is achieved when $MinSup^* = 0.2\%$.

This algorithm is much faster than the 1NN classifier alone. Approximately, the associative classifier localizes 79% of the test data in 30 minutes, and the secondary classifier (i.e.,1NN) takes 8 hours to predict the rest, while 1NN classifier, overally applied on the whole test data, takes 2 days .

Average Recall (%)	Average Precision (%)	Average F-Measure (%)
89.79%	88.31%	89.06

Table 5.9: Summary of the best result from associative-1NN classifier

It is an interesting observation from Figure 5.3(a) that the F-Measure is constant for $1\% \leq MinSup^* \leq 4\%$. After a short study, it was discovered that changing $MinSup^*$ within that interval causes a change in only true positive (TP) and true negative (TN) measures of the two classifiers such that the sum of TP and TN in the combined model is constant. Table 5.10 is a real example showing that none of the measures change in total for supports of 1% and 4%.

As compared to the results of single associative classifier, F-Measure is improved by 9% when it is combined with 1NN classifier.

	MinSup* = 1%					MinSup* = 4%			
	TP	TN	FP	FN		TP	TN	FP	FN
Associative Classifier	30	258	2	0	⇒	29	101	2	0
1NN Classifier	8	743	4	4		9	900	4	4
Total	**38**	**1001**	**6**	**4**		**38**	**1001**	**6**	**4**

Table 5.10: The change in true positive and true negative of individual classifiers for MinSup* = 1% and 4%. Totally, measures are constant.

5.3.6 Combination of SVM and 1NN Classifiers

Unlike associative classifier, SVM predicts a class label for any input vector. To exploit the secondary classifier, only silent proteins (expressed by a zero vector) are excluded from the test data of SVM, and are put aside for the 1NN classifier. Silent proteins are, on average, 21.71% of the test data, that is very close to the percentage of undecided proteins in the case of using associative classifier, which is 21.76%. It means that almost the same proteins are classified by 1NN regardless of whether SVM or associative classifier is the primary predictor. It makes the combination of SVM and 1NN fairly comparable to the combination previously introduced. Table 5.11 summerizes the result of combining 1NN with different SVM classifiers.

	F-Measure		
	Linear Kernel	**Sigmoid Kernel**	**Radial Basis Function kernel**
C = 1	82.12	29.48	29.48
C = 10	**82.48**	29.48	29.48
C = 100	82.34	40.25	52.59
C = 1000	82.34	40.25	81.37
C = 10000	82.34	82.48	81.37

Table 5.11: The result of SVM-1NN classifier. SVM is the primary and 1NN is the secondary classifier

With linear kernel function where the cost is set to 10, the highest F-Measure is achieved which is shown in Table 5.12.

Average Recall (%)	Average Precision (%)	Average F-Measure (%)
77.98	87.61	82.48

Table 5.12: Summary of the best result from SVM-1NN classifier

5.3.7 Combination of Decision Tree and 1NN Classifiers

In a way similar to that for SVM-1NN classifier, only silent proteins are classified by 1NN and the rest by a decision-tree-based classifier, i.e.,ID3 and C4.5. The result of this combination is presented in Table 5.13. According this table, ID3 achieves a higher F-Measure. Like other combinations, adding 1NN classifier to the decision-tree-based classifiers causes an increase of around 9% in the F-Measure.

Algorithm	Recall (%)	Precision (%)	F-Measure (%)
ID3	78.02	87.56	**82.36**
C4.5	72.54	88.85	79.41

Table 5.13: The result of ID3 and C4.5 in combination with 1NN classifier

5.3.8 Comparison of Different Classifiers

According to the experiments discussed aboved, the use of 1NN as a secondary classifier improves the F-Measure of each individual classifier by an approximate increase of 9%. However, other than associative classifier, the algorithms could not outperform single 1NN classifier when they are combined with it. Associative classifier combined with 1NN is the winner algorithm with an F-Measure of 89.06%, approximately 1.23% higher than the F-Measure of single 1NN classifier. Although it is not a big difference, there are still advantages for the combined model. It is much faster than single 1NN classifier. To compare the run time, creating the distance matrix of proteins for the 1NN classifier takes around 52 hours on the machine described in Section 4.2 while creating the PBS-based feature dataset, building an associative classifier and predicting the localization of the test proteins all take at most half an hour. The time for 1NN classification of only 21% of the test data that are undecided should also be considered, which is not analogous to 1NN classification of 100% of the test data. Figure 5.4 compares all the experimented classifiers in terms of F-Measure, the prediction ability. The figure shows

that single associative classifier outperforms single SVM and single decision tree classifiers with a difference of at least 7%.

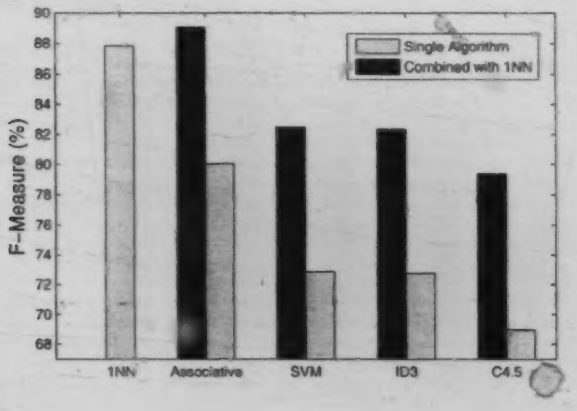


Figure 5.4: The comparison of different models in terms of their prediction accuracy.

5.4 The Reliability of the Parameter Setting Approach For Feature Mining

Earlier in Section 5.2, we speculated that the setting of $MinSup = 0.2\%$ and $MinLen = 7$ for PBSs should later result in the most accurate prediction. Now that associative-1NN classifier is known as the most accurate for this problem, we create other feature datasets, by fixing one of the parameters and varying the other one, and study how the prediction of this winner classifier changes. Figure 5.5 and Figure 5.6 show that the F-Measure of the associative-1NN classifier cannot be higher on other feature spaces, obtained from other feature mining parameter settings.

MaxPart is also an important parameter for feature mining. As we mentioned earlier, we believe that PBSs can better discriminate proteins than frequent simple subsequences. In other words, $MaxPart = 1$, which means no partitioning, should result in the least accurate prediction. Moreover, the prediction is expected

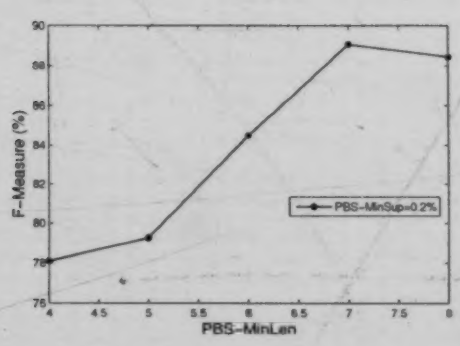


Figure 5.5: With MinSup fixed to 0.2%, initial value 7 for MinLen has been the best setting.

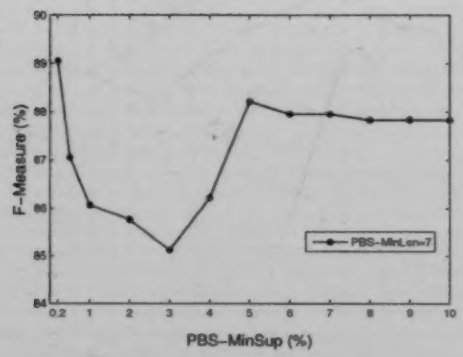


Figure 5.6: With MinLen fixed to 7, initial value 0.2% for MinSup has been the best setting.

to improve by increasing *MaxPart* because it generates more specific PBSs. Experiments show that when long subsequences are mined, e.g.,*MinLen* = 7, exploiting the partitions does not make a remarkable improvement in prediction. It is because of the high length of subsequences that makes them specific enough to their own class. Nonetheless, short subsequences, e.g.,*MinLen* = 4, are more likely to appear in both classes of proteins. The information of where these short subsequences appear in the protein sequence adds specificity to them. Figure 5.7 and Figure 5.8 show how the F-Measure of the associative classifier and the associative-1NN classifier increase when more partitions are considered. The prediction improvement is more sensed when *MinLen* is 4 rather than 7. It should be highlighted that in these figures, there is a big jump after *MaxPart* = 1, where partitiong begins.

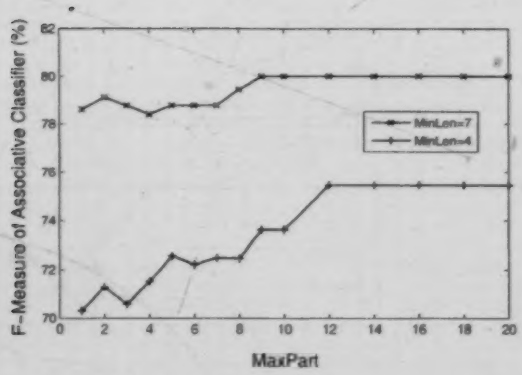


Figure 5.7: The increase of F-Measure in associative classifier by partitioning proteins.

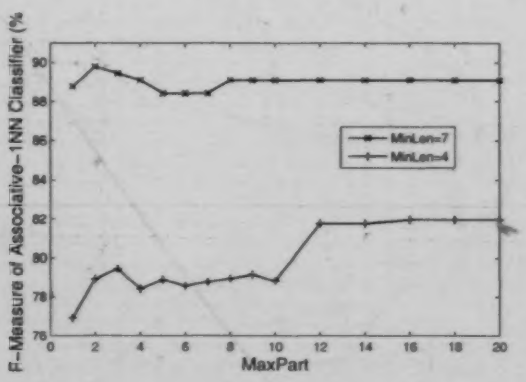


Figure 5.8: The increase of F-Measure in associative-1NN classifier by partitioning proteins.

Chapter 6

Conclusion And Future Work

In this research, we proposed a new discriminative feature for predicting extracellular proteins. Partition-Based Subsequences have a strong ability, higher than simple subsequences, to discriminate between the proteins of different localizations. Moreover, they seem to encode more information about the structure of proteins by showing the regions along the protein sequences where special subsequences appear most. We applied an associative classifier on the feature datasets. With some simple, interpretable and highly confident rules most of proteins are well classified. In a few cases where an associative classifier is not certain about the localization of a protein, a nearest neighbor classifier based on edit distance is utilized. The combination of the associative and the nearest neighbor classifiers is a strong model to predict extracellular proteins with an F-Measure of 89.06%, which is significantly above the state-of-the-art, almost 5% above the F-Measure of Proteome Analyst and the previous work, which is specifically on EC prediction using the same dataset. The presicion of this model is 88.31%, and its recall is 89.79%. Our associative classifier also outperforms SVM and decision tree classifiers such as ID3 and C4.5 on the same feature space with a large difference of at least 7% in the F-Measure.

As one future work, this binary classifier can be extended to a complete localization problem. This can be done hierarchically. In the first step, a decision is made whether a protein is extracellular or intracellular. If it is intracellular, next steps similarly investigate which intracellular location the protein resides in. In each step, one location is specifically learnt while the remainder is called "other" until all the locations are learnt in the end.

Another interesting work is to use another alphabet for representing proteins and extract frequent subsequence-based features in this new representation. There are biochemical similarities among some of the amino acids []. In this context, two or more similar amino acids (with similar properties) can be grouped and represented by a single alphabetic code. It reduces the number of characters in the string represention of proteins, and may generate smaller but more valuable set of features. Therefore, instead of chains of amino acids alone, chains of amino acid groups will be found which might be more meaningful to biologists.

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